DAIRY INGREDIENTS for FOOD PROCESSING

Ramesh C. Chandan and Arun Kilara
Editors

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Dairy Ingredients for Food Processing

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Edited by

Ramesh C. Chandan
Arun Kilara

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Preface

Dairy Ingredients for Food Processing includes advances in technology of major dairy ingredients and their uses in the processing of important food products. The objective of this book is to provide an updated applied reference book for professionals engaged in management, research and development, quality assurance, and manufacturing operations in the food industry. It is a single source that provides basic and practical information to understand and work with dairy-based ingredients. The book is designed to present the topics in a convenient, easy-to-follow format. The intended audience consists of technical personnel in the food industry as well as students and teachers in food science at the university level.

Dairy Ingredients for Food Processing gives a comprehensive description of various dairy ingredients commonly used in food processing operations. The editorial team has assembled 25 authors from the United States, Australia, New Zealand, and the United Kingdom to write the chapters. These contributors represent diverse expertise from academia, industry, and government research institutions. The editors intended to ensure current practical information and scientific accuracy to provide potential reference value to all engaged in the product development, processing, and quality assurance disciplines of the food industry. This book is not meant to be a treatise on the subject but presents the basic and applied information in a single source. The authors have presented the topics in a concise, easily understandable style to enhance usefulness of the book.

Information is conveniently grouped to include basic technology associated with the manufacture of dairy ingredients, especially the parameters that affect their performance and functionality in food systems. The applications of commonly available dairy ingredients in the manufacture of food products such as dairy foods, bakery products, processed cheese, processed meat, chocolate as well as confectionery products, functional foods, and infant and adult nutritional products are covered in some detail. Information is conveniently grouped under 20 chapters by multiple authors to provide an international perspective.

The individuality of the authors' contributions has been protected by the editors to provide both diversity of information and the focus of the authors. The editors have included minor duplication of some material in certain chapters to give readers another perspective on the subject and to maintain continuity and flow of thought of the respective authors. For the convenience of readers, some basic information has been derived from the previously published book *Dairy Processing and Quality Assurance* (Wiley-Blackwell, 2008).

Chapter 1 provides an overview of the technology of dairy ingredients, and serves as a refresher on the subject. Chapter 2 is devoted to chemical, physical, and functional characteristics of dairy ingredients. The microbiological aspects are given in Chapter 3. To facilitate understanding of the origin of dairy ingredients, the principles of dairy processing are summarized in Chapter 4.

Information on concentrated fluid milk ingredients is discussed in Chapter 5. Dry milk ingredients are described in Chapter 6. Other ingredients including casein, caseinates, and milk protein concentrates are dealt with in Chapter 7. Whey-based ingredients are discussed in Chapter 8. Butter and butter products are found in Chapter 9. Natural and process cheese technology and applications are given in Chapters 10 and 11. Enzyme-modified dairy ingredients are discussed in Chapter 12 and fermented dairy ingredients are presented in Chapter 13.

Dairy fermentations have given the food industry novel and natural preservatives for public health safety and extended shelf life of foods. Furthermore, several functional food ingredients have been developed for the food industry from dairy fermentation technologies, which have been described in Chapter 14. The regulatory aspects of dairy ingredients are presented in Chapter 15, whereas their nutritive and health attributes are given in Chapter 16. The use of dairy ingredients in major dairy manufacturing operations is presented in Chapter 17. The applications of dairy ingredients in bakery, snacks, sauces, dressings, processed meats, and functional foods are discussed in Chapter 18. The applications of dairy ingredients in chocolate and confectionery products are presented in Chapter 19, and their use in infant and adult nutritional products is discussed in Chapter 20.

The authors have attempted to support the origin, properties, and functional characteristics of dairy ingredients as well as their applications in the processing of major food products with sound scientific, technological, and engineering principles. The reader should notice a slant toward practical aspects as well.

It is hoped that the contemporary and experience-based information given in *Dairy Ingredients for Food Processing* will appeal to all the professionals in the food industry, including manufacturers of dairy ingredients. In addition, it is hoped that the book will be a useful resource for members of academia engaged in teaching and research in food science areas, regulatory personnel, food equipment manufacturers, and technical specialists engaged in the manufacture of dairy and food products.

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Dairy Ingredients for Food Processing

Chapter 1

Dairy Ingredients for Food Processing: An Overview

Ramesh C. Chandan

Introduction

Dairy ingredients are important players in the formulation of many food products. The addition of familiar dairy ingredients, widely recognized by the consumer as "natural," enhances the odds of success of packaged foods in the marketplace. They generally deliver a consumer-friendly label on the package.

Dairy ingredients are derived from fluid milk in the form of cream, butter, condensed milk, dry milk, cheese, and whey products (Olson and Aryana, 2008, Sodini and Tong, 2006). They provide desirable functionality to foods, such as delivery of key nutrients, water management, fat-holding capacity, emulsification capability, viscosity creation, gel formation, and foam generation. In addition, dairy-based ingredients in liquid, concentrated, or dry form confer desirable attributes of texture and flavor to dairy foods, frozen desserts, puddings, processed meat, cereal products, chocolate confections, infant formulas, and an array of dietetic as well as geriatric drinks and bars. In conventional bakery items, dairy ingredients are used in enriched breads, croissants, milk bread, cakes, cookies, and pastries. Figure 1.1 demonstrates the relationship of milk to major dairy ingredients used for food processing.

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Dairy ingredients contribute several critical characteristics associated with a food product. Caseinates impart emulsifying and stabilizing ability. Whey protein concentrates and isolates give gelling properties and furnish high-quality protein (Kilara, 2008). Similarly, milk protein concentrates provide a base of dietetic products. High-heat nonfat dry milk is reputed to impart water-absorption capacity to baked goods. Lactose-containing dairy ingredients are responsible for desirable brown crust in bread and other bakery items. Enzyme-modified butter and cheese flavor concentrates are used in food products for butter and cheese carry-over. Dairy ingredients are important tools for a food developer to create certain desirable attributes in foods. An understanding of the functional properties of dairy ingredients allows food technologists to use their potential contributions to meet consumer expectations.

Consumer trends, especially in functional foods (Chandan and Shah, 2007) as well as fast and convenience foods, are shaping the development of new products in the marketplace. More recently, market opportunities have been leveraged in nutraceutical beverages for use as tools for weight management, meal replacement, and geriatric nutritional needs using fluid skim milk, nonfat dry milk, milk protein concentrate, and whey protein concentrate. In addition, coffee-based drinks have provided the consumer with a variety of nutritional and functional drinks.

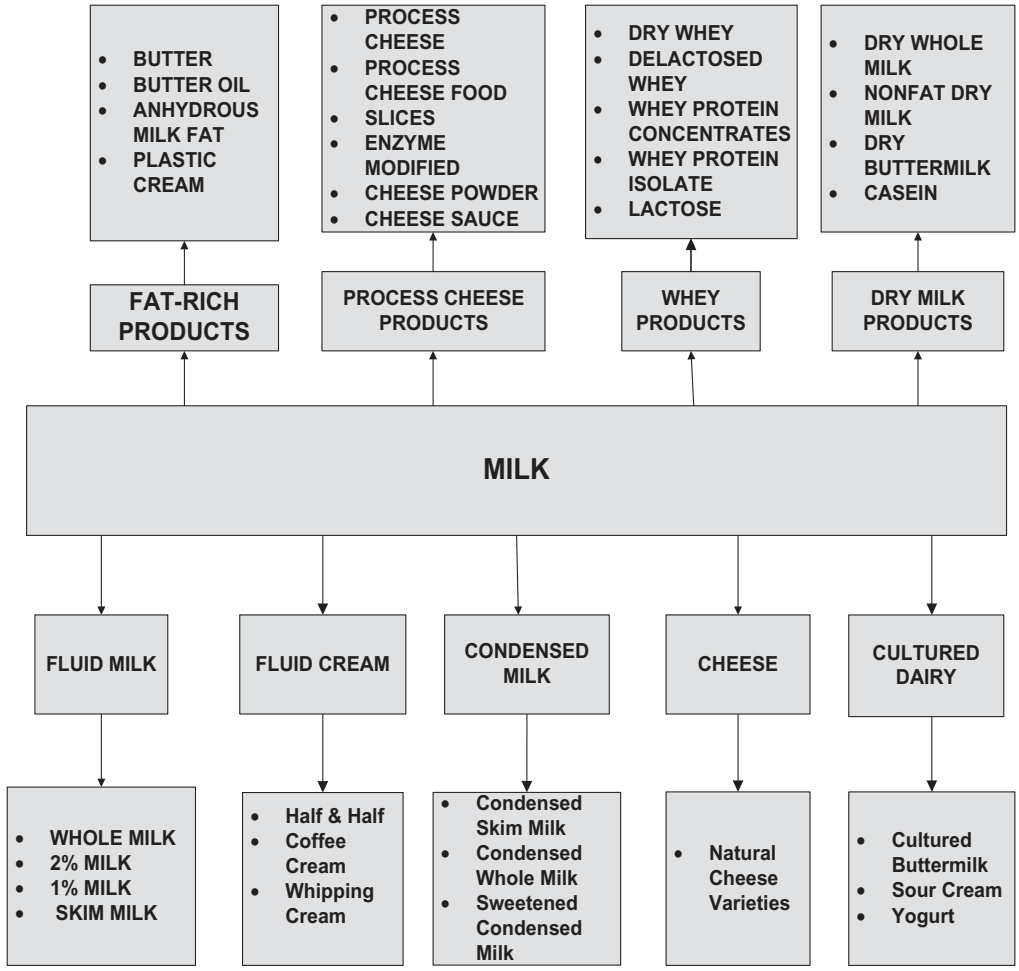

Figure 1.1. Relationship of dairy ingredients to milk.

In the arena of industrial ingredients, dairy plants fabricate convenient, custom-made mixes for food plants for processing of foods. Such practice is currently undertaken for the production of yogurt, ice cream, and confectionery products (Chandan and O'Rell, 2006a; Kilara and Chandan, 2008). Novel ingredients have been developed by applying membrane technology to fractionate milk and whey to enhance their performance in food products. Such ingredients furnish milk protein, milk fat, or milk minerals in food supplements. A new trend involves development of functional ingredients from whey, colostrum, and bioactive peptides from milk proteins, which possess distinct health-promoting attributes (Chandan 2007a and b). Other ingredients are specific metabolites concentrated in fermented milk or whey by the activity of specific dairy cultures. The dried fermented ingredients derived from fermented bases contain active metabolites that are used as natural preservatives to extend shelf life and safety of foods. The enzyme-

modified cheeses are cheese flavor concentrates that are widely used in the production of cheese powders, cheese sauces, and process cheese, and in the preparation of fillings for cookies and crackers.

Milk and Dairy Processing

Fluid milk is a basic ingredient in dairy foods, including frozen and refrigerated desserts (Kilara and Chandan, 2008; Chandan and Kilara, 2008)). Many dairy-derived ingredients for use in food processing owe their origin to milk, which is comprised of water and milk solids. Milk solids are comprised of milk fat and milk-solids-not-fat. Figure 1.2 illustrates the gross composition of milk, showing major constituents. The composition of whole milk solids and nonfat solids is shown in Table 1.1.

Accordingly, incorporation of dairy ingredients in a food adds these constituents to the overall food composition and allows a food developer to leverage their functionality and other attributes in food product development. Chemical, physical, and functional properties of milk are discussed in Chapter 2.

Variations in Milk Composition

It is important to recognize that milk composition varies depending on the breed of the cow, intervals and stages of milking, different quarters of udder, lactation period, season, feed, nutritional level, environmental temperature, health status, age, weather, estrus cycle, gestation period, and exercise (Chandan, 2007a; Kailasapathy, 2008). The variations in major constituents of milk, namely fat, protein, lactose, and minerals, are more noticeable in milk from individual cows. In general, these variations tend to average out and display an interesting pattern in commercial milk used by processors. Nevertheless, the seasonal variations in

Table 1.1. Proximate composition of whole milk solids and skim milk solids.

Component	Whole milk solids	Skim milk/ nonfat solids
Fat, %	29.36	1.08
Protein, casein, %	22.22	31.18
Whey protein, %	4.76	7.53
Lactose, %	38.10	52.15
Ash (minerals), %	5.56	8.06

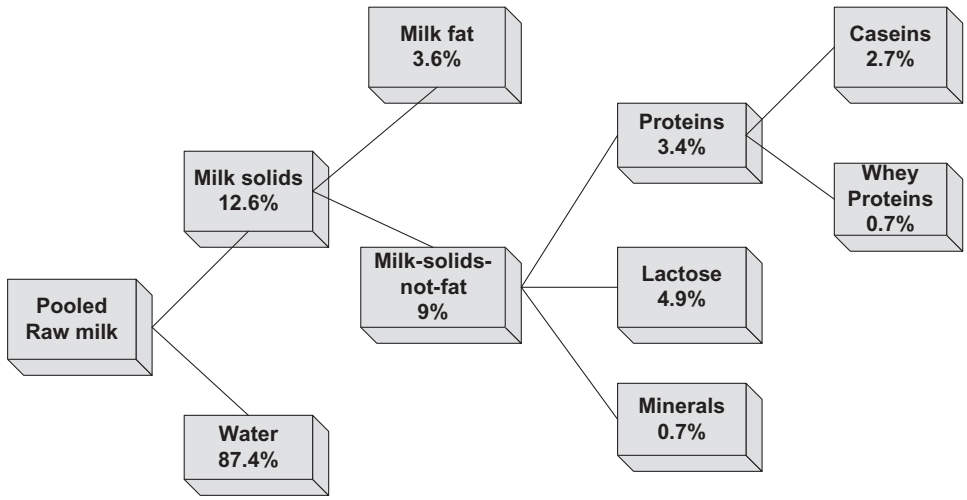

Figure 1.2. Gross composition of pooled raw milk.

major milk constituents still impact important properties of finished products. In the United States, approximately 10% variation in fat and protein is observed in milk received in July and August (lowest level) as compared to milk delivered in October and November (highest level). Subsequently, the functional contribution of milk proteins (viscosity in yogurt and buttermilk, and curd firmness in cheese manufacture) follows a similar trend. Butter produced in summer is generally softer than that produced from winter milk. Furthermore, cheese yield and whey protein production can be negatively affected by seasonal variations in milk composition.

The concentration of minerals such as chloride; phosphates; and citrates of potassium, sodium, calcium, and magnesium in milk is important in processing, nutritive value, and shelf life of dairy products. Their concentration is less than 1% in milk. Still, they affect heat stability of milk, age-thickening of sweetened condensed milk, feathering of coffee cream, rennin coagulation, and clumping of fat globules on homogenization. All of the minerals considered essential for human nutrition are found in milk (Chandan, 2008d). For nutritive and health attributes of dairy ingredients, see Chapter 16.

Important Quality Factors

From a consumer standpoint, the quality factors associated with milk are appearance, color, aroma, flavor, and mouth feel. The color of milk is perceived by the consumer to be indicative of purity and richness. The white color of milk is due to the scattering of reflected light by the inherent ultramicroscopic particles, namely fat globules, colloidal casein micelles, and calcium phosphate. The intensity of white color is directly proportional to the size and number of particles in suspension. Homogenization significantly increases the surface area of fat globules as a result of breakup of larger globules. Accordingly, homogenized milk and cream appear whiter than non-homogenized counterparts. After the precipitation of casein and fat by the addition of a dilute acid or rennet, whey separates out. The whey possesses a green-yellow color due to the pigment riboflavin. The depth of color varies with the amount of fat remaining in the whey. Lack of fat globules gives skim milk a blue tinge. Physiological disturbances in the cow also make the milk bluer.

Cow's milk contains the pigments carotene and xanthophylls, which tend to impart golden yellow color to the milk. Guernsey and Jersey breeds produce especially golden yellow milk. Milk from goats, sheep, and water buffalo tends to be much whiter in color because their milk lacks the pigments.

The flavor of milk is critical to its consumer quality criterion. Flavor is an organoleptic property in which both odor and taste interact. The sweet taste of lactose is balanced against the salty taste of chloride, and both are somewhat moderated by proteins. This balance is maintained over a fairly wide range of milk composition, even when the chloride ion varies from 0.06% to 0.12%. Saltiness can be organoleptically detected in samples containing chloride ions exceeding 0.12% and it becomes marked in samples containing 0.15%. The characteristic rich flavor of dairy products may be attributed to the lactones, methyl ketones, certain aldehydes, dimethyl sulfide, and certain short-chain fatty acids. As lactation advances, lactose declines while chlorides increase, so that the balance is slanted toward "salty." A similar dislocation is caused by mastitis and other udder disturbances. Accordingly, milk flavor is related to its lactose : chloride ratio.

Freshly drawn milk from any mammal possesses a faint odor of a natural scent peculiar to the animal. This is particularly true for the goat, mare, and cow. The cow odor of cows' milk is variable, depending upon the individual season of the year and the hygienic conditions of milking. A strong "cowy" odor frequently observed during the winter months

may be due to the entry of acetone bodies into milk from the blood of cows suffering from ketosis.

Feed flavors in milk originate from feed aromas in the barn; for instance, aroma of silage. In addition, some feed flavors are imparted directly on their ingestion by the animal. Plants containing essential oils impart the flavor of the volatile constituent to the milk. Garlic odor and flavor in milk is detected just one minute after feeding garlic. Weed flavor of chamomile or mayweed arises from the consumption of the weed in mixtures of ryegrass and clover. Cows on fresh pasture give milk with a less well-defined "grassy" flavor, due to coumarin in the grass. A "clovery" flavor is observed when fed on clover pasture, and these taints are not perceptible when dried material is fed. Prolonged ultraviolet radiation and oxidative taints lead to "mealiness," "oiliness," "tallowiness," or "cappy" odor. Traces of copper (3 ppm) exert development of metallic/oxidized taints in milk. Microbial growth in milk leads to off-flavors such as sour, bitter, and rancid. Raw milk received at the plant should not exhibit any off-flavors. Certain minor volatile flavor may volatilized off by dairy processing procedures. Various off-flavors and their origins are summarized in Table 1.2.

Table 1.2. Origins and causes of off-flavors in milk and dairy ingredients.

Origin	Off-flavor	Description	Potential causes
Chemical/ biochemical	Rancid, lipolytic	Soapy, bitter, unclean, blue-cheese-like aroma, strong, foul, lingering aftertaste	Raw milk homogenization, delay in pasteurization after homogenization, raw milk mixed with pasteurized milk
	Oxidized, light- induced	Feathery, tallow, burnt, medicinal, chemical taste	Milk exposed to UV light (sunlight/fluorescent light in dairy cabinet)
Microbiological	Malty	Grapenut flavor, burnt, caramel	Equipment not properly sanitized, milk not cooled promptly to less than 10°C/50°F
	Acid/sour	Tingling/peeling sensation on tongue	Milk stored warm for prolonged period
	Fermented/ fruity	Odor reminiscent of sauerkraut, vinegar, apple, pineapple, and other fruits	Old, refrigerated pasteurized milk, raw milk stored for prolonged time
	Bitter/unclean	Persistent bitter, unpleasant, musty, stale, dirty, spoiled taste	Dirty utensils and equipment, temperature abuse
Absorbed during milk production	Feed	Aromatic, onion, garlic, clover, reminiscent of feed	Feeding cows 0.5 to 3 hours before milking
	Barn-like	Aroma of poorly maintained barn, unclean aftertaste	Poor barn ventilation and accumulated aromatic odors in barn
	Cow-like	Reminiscent of cow breath odor; unpleasant medicinal, chemical aftertaste	Cows afflicted with ketosis/ acetonemia
Processing induced	Cooked	Scorched, sulfur-like, caramelized, sweet flavor	Pasteurization time and temperatures exceeding normal parameters, heat-sterilized milk
	Flat	Lacking full flavor, no flavor	Low total solids content, watered milk
	Foreign	Flavor and aroma not typical of milk	Contamination with cleaning and sanitary chemicals

Adapted from Chandan (1997, 2007a)

Raw Milk Quality Specifications

It is essential to set up stringent specifications for quality maintenance for purchasing milk, The specifications involve several parameters as discussed below.

Standard plate count (SPC) is a measure of the total bacteria count, and measures the overall microbiological quality of milk. High SPC can cause reduced shelf life of the finished product and off flavors from enzyme activity and elevated acidity.

Per Pasteurized Milk Ordinance (USDHHS PMO, 2003), the U.S. Federal Grade A Standards allow a maximum of 100,000 CFU/ml for an individual producer and 300,000 CFU in commingled milk. However, some states differ. For example, for an individual producer, the Idaho standard is 80,000 CFU/ml maximum and the California standard is 50,000 CFU/ml maximum. It is recommended to set the standard at 50,000 CFU/ml.

Coliform bacteria count is a measure of milk sanitation. High coliform counts reflect poor milking practices and unsatisfactory cleanliness of the dairy operation. Occasionally, coliform count may indicate sick cows in smaller herds. Coliform count is an indicator that food poisoning organisms may be present. There are no federal standards for coliform counts in raw milk, but California has a standard for coliform (750 CFU/ml maximum). A recommended standard is 500 CFU/ml.

Laboratory pasteurized count (LPC) is a measure of heat-stable bacteria that may survive pasteurization. It is performed by heat-treating laboratory samples to simulate batch pasteurization at 62.8°C (145°F) for 30 minutes and enumerating the bacteria that survive using the SPC method. High LPC results indicate potential contamination from soil and dirty equipment at the dairy. High LPC causes reduced shelf life of finished products. *Bacillus cereus* is a common soil microorganism that can survive pasteurization, resulting in a high LPC. There are no federal standards for LPC. However, the California standard for LPC is 750 CFU/ml maximum. A recommended standard is 500 CFU/ml.

Preliminary incubation (PI) count is a measure of bacteria that will grow in refrigerated conditions. The test requires holding the sample at 10°C (50°F) for 18 hours followed by a SPC test. PI type of bacteria are destroyed by pasteurization but can still result in lower quality milk due to enzymatic activity on the protein. High PIs (3- to 4-fold higher than SPCs) are generally associated with inadequate cleaning and sanitizing of either the milking system or cows and/or poor milk cooling.

There are no federal standards for PI counts in raw milk. Because the type of bacteria and the initial count of the SPC may vary, it is not possible to set a numerical standard for this test. A recommended standard is less than two times the SPC count.

Somatic cell count (SCC) is a measure of the white blood cells in the milk. It is used as an indicator of herd health. High SCCs are undesirable because the yield of all cultured products is proportionally reduced, the flavor becomes salty, development of oxidation increases, and it usually relates to higher SPC. Staphylococci and streptococci are heat-tolerant bacteria that normally cause mastitis. Coliform bacteria, which are easily killed by heat, may cause mastitis. The PMO standards allow individual milk not to exceed 750,000 cells/ml. State standards vary. For example, the California standard is 600,000 cells/ml maximum. A recommended standard is 500,000 cells /ml.

Titratable acidity (TA) is a measure of the lactic acid content of milk. High bacteria counts produce elevated lactic acid levels as the bacteria ferment lactose. The normal range of TA in fresh milk is 0.13% to 0.16%. Elevated temperatures for an extended time allow the bacteria to grow and generate a higher TA value. Lower values

may indicate the presence of chemicals in the milk. A recommended standard is 0.13% to 0.17% TA.

Temperature According to the PMO standard, the temperature of milk must never exceed 7°C (45°F). A recommended standard is 5°C (40°F) or less.

Flavor is an important indicator of quality, as stated earlier. The milk should be fresh and clean with a creamy appearance. Elevated bacteria counts can produce off-flavors (for example, acid, bitter). Feed flavors may vary from sweet to bitter and indicate the last items in a cow's diet, such as poor feed, weeds, onion, or silage. Elevated somatic cell counts make milk taste salty and watery. Water in the milk gives it a watery taste. Dirty, "barny," and "cowy" flavors occur from sanitation conditions and air quality at the dairy farm. Oxidized or rancid flavors occur from equipment operation and handling.

There are no federal standards for flavor. All receiving plants should flavor milk for defects before accepting it.

A recommended standard is that no off-flavor exists.

Appearance is not a measured criterion but for indications of quality it is as important as flavor. There are no federal standards for appearance. Most receiving plants must note any color or debris defect in the milk before accepting it. A recommended standard is "White, clean, no debris, and filter screen of 2 or less (sediment test)."

Antibiotics and other drugs may not be present in milk. All raw milk must conform to the PMO Grade A regulations (Frye, 2006). To be considered organic, no milk can be used from a cow that has been treated with antibiotics without a 12-month holding period following treatment. For conventional milk, a treated cow will be withheld from the milking herd for about 5 days.

Added water is an adulteration. Testing the freezing point of milk using a cyroscope indicates if abnormal amounts of water exist in the load. In most states it is illegal to have a freezing point above –0.530° Hortvet scale. A recommended standard should be –0.530° Hortvet or less.

Sediment is measured by drawing 1 pint of sample through a cotton disk and assigning a grade of 1 (good) to 4 (bad) to the filter. A grade of 1 or 2 is acceptable. A processor also may monitor for sediment by screening the entire load through a 3-inch mesh filter at the receiving line. There are no federal standards. Most receiving plants should require a filter grade of 1 or 2, although a 3 may be accepted.

A recommended standard is "No excessive material in a 3-inch sani-guide filter."

Fat and milk-solids-not-fat (MSNF) have FDA standards of identity for milk of 3.25% fat and 8.25% MSNF. This is the recommended standard.

In the recent past, major advances in dairy processing have resulted in improvement in safety and quality of products. In particular, ultra-pasteurization techniques and aseptic packaging systems have presented the industrial user with extended and long shelf-life products.

Basic Steps in Milk Processing

It is beneficial for food developers and processors to know the basic steps involved in dairy processing. A detailed description of basic dairy processing is given in Chapter 4. Milk production, transportation, and processing are regulated by Grade A Pasteurized Milk Ordinance (USDHHS PMO, 2003; Frye and Kilara, 2006). Chapter 15 of this book deals with the regulatory aspects of dairy-based ingredients. Figure 1.3 shows the journey of milk from the farm to supermarket, including processing at the milk plant.

Bulk Milk Handling and Storage

The handling and storage of bulk milk are key components of good quality milk. Dairy

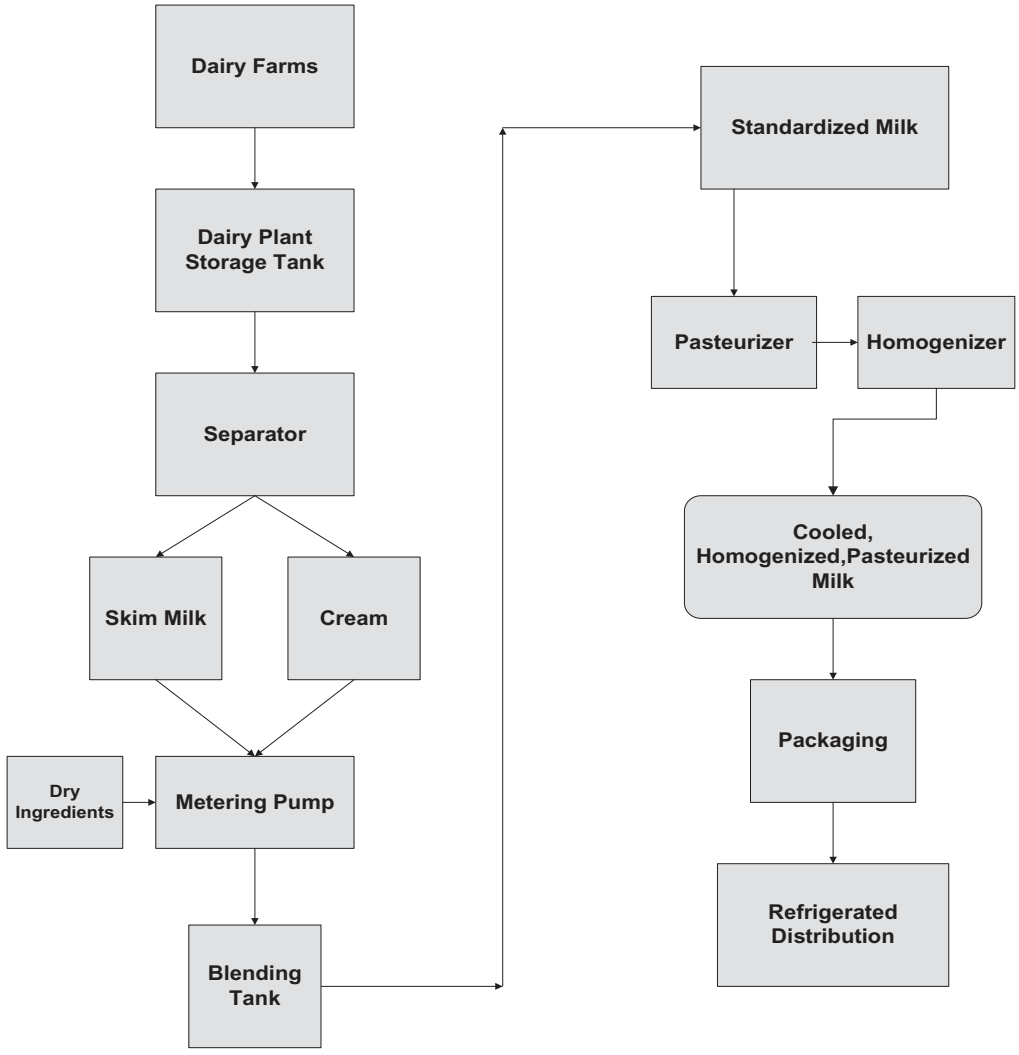

Figure 1.3. The journey of milk from farm to market.

farms produce sanitary raw milk under the supervision of U.S. Public Health Services (Pasteurized Milk Ordinance). The regulations help in the movement of assured quality milk across interstate lines.

Today, virtually all the raw milk at the plant is delivered in tank trucks. Unloading of milk involves agitation of the truck, inspection for the presence of off-flavors, collection of a representative sample, and connection of the unloading hose to the truck outlet. After opening the tank valve, a high-capacity transfer pump is used to pump milk to a storage tank or silo. The weight of milk transferred is registered with a meter or load cells. The tank truck is then cleaned by plant personnel by rinsing with water, cleaning with detergent solution, rinsing again with water, and finishing with a chlorine/iodine sanitizing treatment. A clean-in-place line

may be inserted into the tank through the manhole. Payment of milk is based on the hauler receipt.

Storage tanks may be refrigerated or insulated. They hold milk up to 72 hours (usually 24 hours) before processing. The tanks may be horizontal or vertical in configuration. Grade A milk for pasteurization must be stored at 1.7°C to 4.4°C (35°F to 40°F). The maximum bacterial count at this stage is 300,000 CFU/ml, as opposed to the maximum of 100,000 CFU/ml allowed at the farm. The higher count is justified because pumping breaks the clumps of bacteria, which gives higher counts and provides more opportunity for contamination of milk as it comes in contact with more equipment during handling and transfer. Also, the longer storage time adds more bacterial numbers. The 3-A sanitary standards are followed for equipment design (Frye, 2006). Chapter 3 deals with the microbiological aspects of milk and dairy ingredients.

Separation

The purpose of the separation step is to separate milk into cream and skim milk. All incoming raw milk is passed through the separator, which is essentially a high-speed centrifuge. This equipment separates milk into lighter cream fraction and heavier skim milk fraction. A separator of adequate bowl capacity collects all the "slime" material containing heavy casein particles, leukocytes, larger bacteria, body cells from cows' udders, dust and dirt particles, and hair. Homogenized milk develops sediment upon storage if this particulate fraction of raw milk is not removed. Skim milk and cream are stored separately for further processing.

Standardization

Use of a separator also permits fractionation of whole milk into standardized milk (or skim milk or low-fat milk) and cream. Skim milk should normally contain 0.01% fat or less. A standardization valve on the separator permits the operator to obtain separated milk of a predetermined fat content. Increased back pressure on the cream discharge port increases the fat content in standardized milk. By blending cream and skim milk fractions, various fluid milk and cream products of required milk fat content can be produced.

Heat Treatment

The main purpose of heat treatment of milk is to kill 100% of the disease-producing (pathogenic) organisms and to enhance its shelf life by removing approximately 95% of all the contaminating organisms. Heat treatment is an integral part of all processes used in dairy manufacturing plants. Intensive heat treatment brings about interactions of certain amino acids with lactose, resulting in color changes in milk (Maillard browning) as observed in sterilized milk and evaporated milk products.

Among milk proteins, caseins are relatively stable to heat effects. Whey proteins tend to denature progressively by severity of heat treatment, reaching 100% denaturation at 100°C (212°F). In the presence of casein, denatured whey proteins complex with casein, and no precipitation is observed in milk. In contrast to milk, whey that lacks casein, and heat treatment at 75°C to 80°C (167°F to 176°F) results in precipitation of the whey proteins.

From a consumer standpoint, heat treatment of milk generates several sensory changes (cooked flavor) depending on the intensity of heat. In general, pasteurized milk possesses the most acceptable flavor. Ultra-pasteurized milk and ultra-high-temperature (UHT) milk exhibit a slightly cooked flavor. Sterilized milk and evaporated milk possess a pronounced cooked flavor and off-color.

The U.S. Food and Drug Administration (PMO) has defined pasteurization time and

temperature for various products. The process is regulated to assure public health. Milk is pasteurized using plate heat exchangers with a regeneration system. The process of pasteurization involves heating every particle of milk or milk product in properly designed and operated equipment to a prescribed temperature and holding it continuously at or above that temperature for at least the corresponding specified time. Minimum time-temperature requirements for pasteurization are based on thermal death time studies on the most resistant pathogen that might be transmitted through milk. Table 1.3 gives the various time-temperature requirements for legal pasteurization of dairy products.

Most refrigerated cream products are now ultra-pasteurized by heating to 125°C to 137.8°C (257°F to 280°F) for two to five seconds and packaged in sterilized cartons in clean atmosphere. For ambient storage, milk is UHT treated at 135°C to 148.9°C (275°F to 300°F) for four to 15 seconds, followed by aseptic packaging. In some countries, sterilized/canned milk is produced by a sterilizing treatment of 115.6°C (240°F) for 20 minute. It has a light brown color and a pronounced caramelized flavor.

Homogenization

Homogenization reduces the size of fat globules of milk by pumping milk at high pressure through a small orifice, called a valve. The device for size reduction, the homogenizer, subjects fat particles to a combination of turbulence and cavitation. Homogenization is carried out at temperatures higher than 37°C (99°F). The process causes splitting of original fat globules (average diameter approximately 3.5 μm) into a very large number of much smaller fat globules (average size less than 1 μm). As a consequence, a significant increase in surface area is generated. The surface of the newly generated fat globules is then covered by a new membrane formed from milk proteins. Thus, the presence of a minimum value of 0.2 g of casein/g fat is desirable to coat the newly generated surface area. As milk is pumped under high pressure conditions, the pressure drops, causing breakup of fat particles.

If the pressure drop is engineered over a single valve, the homogenizer is deemed to be a single-stage homogenizer. It works well with low-fat products or in products in which high viscosity is desired, as in cream and sour cream manufacture. On the other hand, homogenizers that reduce fat globule size in two stages are called dual-stage homogenizers. In the first stage the product is subjected to high pressure (for example, 13.8 Mpa, 2,000 psi) which results in breakdown of the particle size diameter to an average of less than 1 μm. Then the product goes through the second stage of 3.5 MPa (500 psi) to break the clusters of globules formed in the first stage. Dual stage homogenization is appropriate for fluids with high fat and solids-not-fat content or whenever low viscosity is needed.

Homogenized milk does not form a cream layer (creaming) on storage. It displays a whiter color and fuller body and flavor characteristics. Homogenization leads to better viscosity and stability by fully dispersing stabilizers and other ingredients in ice cream, cultured products, and other formulated dairy products.

Cooling, Packaging, and Storage

Pasteurized fluid milk products are rapidly cooled to less than 4.4°C (40°F), packaged in appropriate plastic bottles/paper cartons, and stored in cold refrigerated rooms for delivery to grocery stores or warehouses for distribution.

Fluid Milk Products

Commercial milk is available in various milk fat contents. The approximate composition of fluid milk products is shown in Table 1.4. The

Table 1.3. Minimum time-temperature requirements for legal pasteurization in dairy operations.

Process	Milk: whole, low fat, skim/nonfat	Milk products with increased viscosity, added sweetener, or fat content 10% or more	Eggnog, frozen dessert mixes
Vat (batch)	30 minutes at 63°C(145°F)	30 minutes at 66°C (150°F)	30 minutes at 69°C (155°F)
High temperature short time	15 seconds at 72°C (161°F)	15 seconds at 75°C (166°F)	25 seconds at 80°C (175°F) 15 seconds at 83°C (180°F)
Higher heat Shorter time	1 second at 89°C (191^{O}F) 0.5 second at 90°C (194°F) 0.1 second at 94°C (201°F) 0.05 second at 96°C (204°F) 0.01 second at 100°C (212°F)	1 second at 89°C(191°F) 0.5 second at 90°C(194°F) 0.1 second at 94°C (201°F) 0.05 second at 96°C (204°F) 0.01 second at 100°C(212°F)	1 second at 89°C (191°F) 0.5 second at 90°C (194°F) 0.1 second at 94°C (201°F) 0.05 second at 96°C (204°F) 0.01 second at 100°C (212°F)
Ultra pasteurized	2 seconds at 138°C (280.4°F)	2 seconds at 138°C (280.4°F)	2 seconds at 138°C (280.4°F)
Ultra-high temperature (UHT), aseptic	Comply with low acid canned food regulations (21CFR 113)	Comply with low acid canned food regulations (21CFR 113)	Comply with low acid canned food regulations (21CFR 113)

Adapted from Chandan (1997), Partridge (2008), USHHS FDA (2003)

Table 1.4. Typical composition of fluid dairy ingredients.

Dairy Ingredient	% Water	% Fat	% Protein	% Lactose	% Ash
Whole milk	87.4	3.8	3.2	4.9	0.7
Skim milk	90.9	0.1	3.3	5.0	0.7
Half and half	80.2	11.5	3.1	4.5	0.7
Light cream	74.0	18.3	2.9	4.2	0.6
Light whipping cream	62.9	30.5	2.5	3.6	0.5
Heavy whipping cream	57.3	36.8	2.2	3.2	0.5
Plastic cream	18.2	80.0	0.7	1.0	0.1
Fluid UF* whole milk	70–75	11–14	10–12	<5	>2.5
Fluid UF* skim milk	80–85	<0.5	10–12	<5	>2.5
Fluid UF* skim milk, diafiltered	80–82	<0.5	16–17	<1	>1.5

*UF, ultra-filtered
Adapted from Chandan (1997), Chandan and O'Rell (2006a)

term "milk" is synonymous with whole milk, which must contain not less than 3.25% milk fat and 8.25% solids-not-fat. Addition of vitamins A and D is optional. If the vitamins are added, vitamin A must be present at a level of not less than 2,000 IU/quart and vitamin D must be present at 400 IU/quart.

Fat-reduced milks are labeled according to their contribution of grams of fat per reference amount (RA) of 240 ml. Low-fat milk contributes less than 3 g fat per RA, whereas nonfat milk contributes less than 0.5 g of fat per RA. Because 2% milk contributes 4.8 g fat/RA, it is labeled reduced-fat milk. For a detailed discussion of fluid milk products, see Partridge (2008).

Figure 1.4 shows the steps in production of fluid milk and cream products. The figure shows general processes for manufacture of whole milk, reduced-fat milk, low-fat milk, and skim milk. It also shows how cream and other fluid products are made.

The shelf life of milk is a function of the microbial quality of raw milk, temperature, and time of exposure during storage and handling, pasteurization conditions, equipment sanitation, packaging conditions, and subsequent distribution practices. Fluid milk products display maximum keeping quality when stored at temperatures close to the freezing point (4°C/39.2°F). Let us assume the shelf life of pasteurized milk is 40 days at the storage temperature of 0°C (32°F). It has been demonstrated that the shelf life is shortened to 20 days by storage at 2°C (35.6°F), 10 days at 4°C (39.2°F), 5 days at 7°C (44.6°F), and progressively to fewer days at higher temperatures. This illustration underscores the importance of maintaining refrigerated storage temperature as low as possible to achieve the maximum shelf life of milk.

Ultra-pasteurized products are packaged in a near-aseptic atmosphere in pre-sterilized containers and held refrigerated to achieve an extended shelf life. When an ultra-pasteurized product is packaged aseptically in a specially designed multilayer container, it displays a shelf life longer than any other packaged fluid milk and cream products. UHT products subjected to aseptic heat treatment and packaged aseptically in specially designed multilayer containers can be stored at ambient temperatures for several months.

Fluid Cream

Cream is prepared from milk by centrifugal separation. Heavy cream contains not less than 36% fat and may be called heavy whipping cream. Light whipping cream contains 30% or more milk fat, but less than 36% milk fat and may be labeled as whipping cream. Light cream, coffee cream, or table cream contains not less than 18% milk fat, but less than 30% milk fat. Half and half is normally a blend of equal proportion of milk and

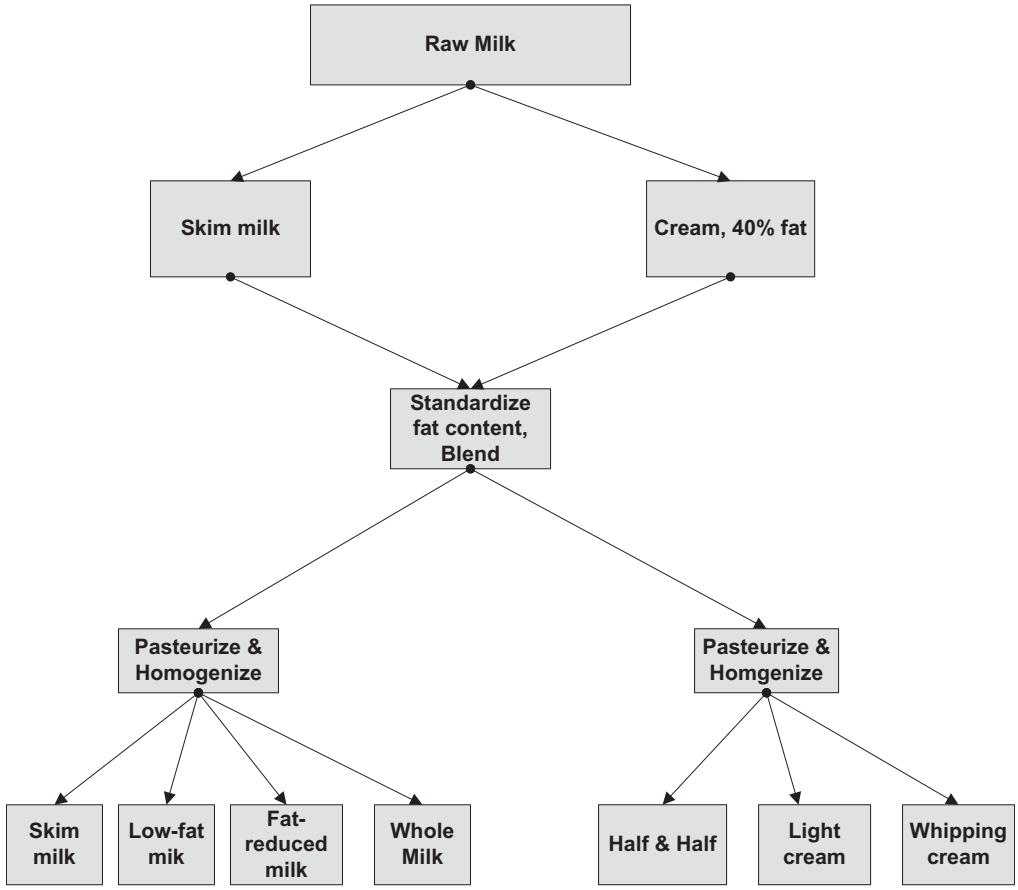

Figure 1.4. Processes for fluid milk and cream.

cream, containing 10.5% milk fat. Legally, it contains not less than 10.5% milk fat but not more than 18% milk fat. Cream to be used as an ingredient in processing contains 36% to 40% fat. Cream of different fat levels can be produced by standardizing with skim milk. Light cream and half and half are homogenized products. Specific homogenization and heat treatments generate desirable grades of viscosity in cream products. They are processed and packaged similar to fluid milks.

Plastic cream contains 80% milk fat. It resembles butter in consistency but compared to butter, it is still oil-in-water type emulsion. As an ingredient, it can be stored in frozen form.

Fat-rich Products

Butter

The manufacture of butter and spreads is discussed in another publication (Fearon and Golding, 2008) and in Chapter 9 of this book. Butter is a concentrated form of milk fat, containing at least 80% fat. It can be converted to shelf-stable products such as butter oil, anhydrous milk fat, and ghee. Table 1.5 shows the approximate composition of butter and its products.

Figure 1.5 is flow-sheet diagram for the manufacture of butter, butter oil, and certain dry milk products. The diagram also displays interrelationships between these products.

Table 1.5. Typical composition of milk fat concentrates.

Product	% Water	% Fat	% Protein	% Lactose	% Ash	Added ingredient
Butter	16.5	80.5	0.6	0.4	2.5	0–2.3% salt
Anhydrous milk fat	0.1	99.8	0.1	0	0	0
Butter oil	0.3	99.6	0.1	0	0	0
Ghee	<0.5	99–99.5	0	0	0	0

Adapted from Chandan (1997), Aneja et al. (2002)

Figure 1.5. Flow sheet diagram for butter, butter oil, dry buttermilk, nonfat dry milk, and dry whole milk.

Butter is obtained by churning cream. The temperature of churning is an important parameter to follow. The churning temperature is determined by an optimum ratio of crystalline fat, solid fat, and liquid fat. The churns are either batch type or continuous type. For batch-type churns, cream of 35% to 45% fat is used. For continuous type churns, cream of 42% to 44% fat is used. Cream is pasteurized at 73.8°C (165°F) for 30 minutes or at 85°C (185°F) for 15 seconds and is then cooled to about 7°C (45°F) for crystallization of fat. The crystallization process is completed by holding the cream for approximately 16 hours.

The cream that registers an increase in temperature to 10°C (50°F) is then transferred to a sanitized churn. Annatto coloring may be incorporated, if required. The churn is continuously rotated to convert oil-in-water type of emulsion (cream) to water-in-oil type emulsion (butter). This conversion is known as phase inversion. This is accompanied by the appearance of butter granules of the size of popcorn or peas. Cream begins to foam during phase inversion. Free fat generated by rupture of fat globules of cream cements some of the remaining fat globules to form clumps or butter granules. There is a clear separation of butter granules from the surrounding liquid, called buttermilk. At this stage, the buttermilk is drained out, followed by the addition of an aliquot of clean cold water (1°C to 2°C/33.8°F to 35.6°F) to the churn. The total volume of wash water is equal to the volume of buttermilk. The washing continues until the rinse is almost clear. Salt at 1.6% level is added and blended with butter. The next step is called "working," in which the remaining fat globules are disrupted to liberate free fat.

All of the free fat then forms the continuous phases in which water droplets are dispersed to form butter. Working of butter is accomplished by continuous rotation of the churn until the body of butter is closely knit to show a waxy character with no visible pockets of surface moisture. The working of butter is continued to standardize moisture until the fat content of butter is 80%. Butter is then pumped and packaged.

Continuous butter churns are now widely in use. They accelerate the churning process, and washing of butter is not necessary. Cream of 42% to 44% fat is introduced into a cylinder, where it is churned. Buttermilk is drained and butter granules are worked to obtain the typical waxy body and texture of butter, followed by packaging. In another process, cream is separated to get plastic cream of 80% fat. The phase inversion is carried out by chilling. The butter granules are worked to achieve typical butter body and texture.

In some countries, butter is churned from cultured cream. Cultured cream butter has a distinct flavor and can be easily distinguished from sweet cream butter.

The processing conditions affect the physical properties such as crystallization and melting behavior of butterfat. The crystal formation is mediated by nucleus formation and subsequent growth of crystals. The size of crystals depends on rate of crystallization. Melting behavior influences the application of butter in food products. The rate of transformation of solid fat fraction into liquid milk fat is important and is characterized by melting point range, thermal profile, and solid fat content. The melting point temperature is the temperature at which milk fat melts completely to a clear liquid. It occurs at a range of 32°C to 36°C (90°F to 97°F) and assumes completely liquid state at 40°C (104°F). It acquires completely solid state at −75°C (−103°F). At ambient temperature, it is a mixture of crystals and liquid phases.

By manipulating temperature, butterfat has been fractionated into three fractions exhibiting distinct functionalities. Low-melting fraction melts below 10°C (50°F), middle-melting fraction melts between 10°C and 20°C (50°F and 68°F), and high-melting fraction melts above 20°C (68°F). Low-melt fraction contains significantly lower levels of saturated fatty acids. Butter made with very low-melt fraction spreads at refrigerated

temperature. Further fractionation leads to very high-melting fraction that melts at a temperature higher than 50°C (above 122°F), behaving like cocoa butter in confectionery products.

Light/reduced fat butter contains 40% fat. The reduced fat form cannot be used for baking.

Butter-vegetable oil blends are obtained by blending certain vegetable oils such as corn oil or canola oil emulsified into cream prior to the churning process. The objective is to reduce the saturated fatty acid content to enhance the healthy perception of the product or to make the product easily spreadable at refrigeration temperature.

Butter oil is at least 99.6% fat and contains less than 0.3% moisture, and traces of milk solids-not-fat. Butter is melted by heating gently to break the emulsion and centrifuged in a special separator to collect milk fat, followed by vacuum drying.

Anhydrous milk fat or anhydrous butter oil is obtained from plastic cream of 70% to 80% fat. Phase inversion takes place in a special unit (separator) and the moisture is removed by vacuum drying. It contains at least 99.8% milk fat and no more than 0.1% moisture.

Ghee is another concentrated milk fat that is widely used in tropical regions of the world, especially in South Asian countries. It is a clarified butterfat obtained by desiccation of butter at 105°C to 110°C (221°F to 230°F). The intense heat treatment generates a characteristic aroma and flavor brought about by heat-induced interactions of components of milk solids of butter. The detailed manufacturing procedure for ghee is given elsewhere (Aneja et al., 2002).

Concentrated/Condensed Fluid Milk Products

For a detailed description of condensed milk and dry milks, see the publications of Farkye (2008) and Augustin and Clarke (2008), and Chapters 5 and 6 of this book. An outline for manufacturing dry whole milk, nonfat dry milk, and dry buttermilk powder is depicted in Figure 1.5. The functional properties of concentrated milk products including nonfat dry milk can be manipulated by specific heat treatment. It also affects the keeping quality of whole milk powder. The temperature and time combinations can vary widely depending on the required functional properties. Invariably, the milk for manufacture of concentrated milk products is pasteurized (high-temperature, short-time) by heating to at least 72°C (161°F) and holding at or above this temperature for at least 15 seconds. An equivalent temperature-time combination can be used. With condensed milk and nonfat dry milk, the extent of heat treatment can be measured by the whey protein nitrogen index, which measures the amount of undenatured whey protein.

Removal of a significant portion of water from milk yields a series of dairy ingredients. Consequently, these ingredients offer tangible savings in costs associated with storage capacity, handling, packaging, and transportation. The composition of concentrated milk products is shown in Table 1.6.

Concentrated milk or condensed whole milk is obtained by removing water from milk and contains at least 7.5% milk fat and 25.5% milk solids. Condensed milk is available in whole milk, low-fat, and nonfat varieties. Condensed whole milk is purchased largely by confectionary industries. It is pasteurized but not sterilized by heat. It may be homogenized and supplemented with vitamin D.

Condensed skim milk is commonly used as a source of milk solids in dairy applications and in the manufacture of ice cream, frozen yogurt, and other frozen desserts. Condensed milks are generally customized orders. User plants specify total solids concentration, fat level, heat treatment, and processing conditions. The dairy concentrates offer economies of transportation costs and

Table 1.6. Typical composition of condensed milk products.

Products	% Water	% Fat	% Protein	% Lactose	% Ash	Added ingredient
Sweetened condensed whole milk	26.1	8.7	7.9	11.3	1.8	44.2% sucrose
Sweetened condensed skim milk	28.4	0.3	10.0	16.3	2.3	42.7% sucrose
Condensed whole milk	74.5	7.5	6.2	9.4	1.6	
Condensed skim milk—medium solids	70.0	0.4	10.8	15.5	2.3	
Condensed skim milk—high solids	59.9	0.4	14.4	22.3	3.0	
Evaporated whole milk	74.0	7.6	6.8	10.0	1.6	
Evaporated low-fat milk	79.0	0.2	7.6	11.4	1.8	

Adapted from Chandan (1997)

storage space. They must be transported and stored at 4.4°C (40°F), and to preserve quality they are used within five days.

Depending on the end user requirements, raw milk is standardized to desired milk fat : nonfat solids ratio. In general, the original milk volume is reduced to about one-third to yield about 25% to 40% solids in the final product. The standardized milk is preheated to 93.3°C (200°F) and held for 10 to 20 minutes. The objective of preheat treatment is to destroy microorganisms and enzymes and to increase heat stability of the milk. In addition, the viscosity of condensed milk is controlled by a time-temperature regime during preheat treatment. The heated milk is concentrated in energy-efficient multi-effect evaporators that operate in high vacuum condition to boil off water at moderate temperatures of 46.1°C to 54.4°C (115°F to 130°F). The concentrated milk is continuously separated from water vapor to achieve desirable concentration of milk solids. It may be homogenized prior to cooling and packaging or pumped to insulated trucks for transportation to user plants.

Sweetened condensed milk contains 60% sugar in the water phase, which imparts a preservative effect. Consequently, it has enhanced shelf life. When packaged properly, the product is stable for many months at ambient storage temperature. Because it does not need high heat treatment for sterilization, it possesses a much better color and flavor than evaporated milk. Condensed milk may be low fat and nonfat. It is derived from milk after the removal of 60% of its water. It must contain at least 8% milk fat and 28% milk solids. The viscosity of the product is high, approximating 1,000 times that of milk. Sweetened condensed milk is used in confectionery manufacture as well in the manufacture of exotic pies and desserts.

Manufacture of sweetened condensed milk resembles the manufacture of condensed skim milk given above. The addition of sugar and control of lactose crystal size require special processing procedures. The standardized milk is preheated at 135°C (275°F) for 5 seconds or 110°C to 120°C (230°F to 248°F) for 10 to 20 seconds. The ultra-heat treatment is preferred over high-temperature-short-time treatment because it leads to lower viscosity in sweetened condensed milk. Following homogenization at 70°C (158°F) at 3.5 MPa (500 psi), milk is concentrated in an efficient evaporator at 82.2°C (180°F) and liquid sucrose is blended. At this stage, the mix is standardized to 8.5% fat, 20% nonfat solids, and 44% sucrose. The blend is then pasteurized at 82.2°C (180°F) for 30 seconds and further standardized to desirable solids in the finishing pan. The product is cooled to 60°C (140°F), followed by seeding with finely ground lactose at the rate of 0.03% (dry matter basis). At this stage the mixture is agitated vigorously while cooling to 18.3°C (65°F).

The lactose crystal size must be less than 10 µm to avoid settling in storage and to prevent sandiness in the product. Sweetened condensed milk is packaged in metal or plastic containers and sealed. For bulk sales, it is pumped into insulated trucks for transport and delivery to user plants.

Evaporated milk is also concentrated milk that is homogenized and heat sterilized in sealed cans or bottles. It is made by boiling off 60% of the water content of milk. It must contain at least 6.5% milk fat and 23% milk solids. Evaporated milk is heat-sterilized. The sterilization process renders the product safe for consumption and it can be stored at room temperature for several months without deterioration of flavor. The current processing trend is to subject the product to ultra-heat treatment, followed by aseptic packaging. This process gives a product with better color and flavor than the in-can sterilized product. Typically, the concentration factor is of the order of 2.1 times, giving a milk fat level of approximately 8% and nonfat solids of approximately 18%. Low-fat evaporated milk composition is 4% fat and 20% nonfat solids, whereas nonfat evaporated milk contains 0.1% fat and 22% nonfat solids. Evaporated milk is mainly a retail canned product used by the consumer as a convenience ingredient in the preparation of meals, snacks, and desserts.

Manufacture of evaporated milk involves standardization of milk to a desired fat : nonfat solids ratio and preheating to 135°C (275°F) for 30 seconds. The milk is concentrated in a vacuum evaporator at 68.3°C to 82.2°C (155°F to 180°F) and homogenized at 65°C (14°F) and 20.7 MPa (3,000 psi), first stage, and 3.5 MPa (500 psi), second stage. It is then cooled to 10°C (50°F) and stabilized with disodium hydrogen phosphate to reduce age thickening during subsequent storage. The product is packaged in metal cans and sealed, followed by sterilization at 120°C (248°F) for 15 minutes.

In a more recent process, the product is vacuum-concentrated and stabilized with disodium hydrogen phosphate as in the conventional process. It is then sterilized at 140.6°C (285°F) for 15 seconds, cooled to 60°C (140°F), and homogenized at 41.3 MPa (6,000 psi). After cooling to 10°C (50°F), evaporated milk is packaged aseptically in appropriate containers.

Dry Milk Products

Table 1.7 gives the typical composition of dry milk products.

Nonfat dry milk (NFDM) is the product resulting from the removal of fat and water from milk. It contains the lactose, milk proteins, and milk minerals in the same relative proportions as in the fresh milk from which it was made. It contains no more than 5% moisture by weight. The fat content does not exceed 1.5% by weight unless otherwise indicated. NFDM is used in dairy products, bakery goods, dry mixes, chemicals, and meat processing, and in homes for cooking.

NFDM is manufactured by spray drying condensed skim milk. Spray drying involves atomizing concentrated milk into a hot air stream 180°C to 200°C (356°F to 392°F). The atomizer may be a pressure nozzle or a

Table 1.7. Typical composition of dry milk products.

Products	% Water	% Fat	% Protein	% Lactose	% Ash
Dried whole milk	3.0	27.5	26.4	37.2	5.9
Nonfat dry milk	3.2	0.8	36.0	52.0	8.0
Dried buttermilk	3.0	5.3	32.4	51.3	8.0
Spray-dried cream (from 20% cream)	0.6	71.1	11.1	14.7	2.5

Adapted from Chandan (1997), Chandan and O'Rell (2006a)

centrifugal disc. By controlling the size of the droplets, the air temperature, and the airflow, it is possible to evaporate almost all the moisture while exposing the solids to relatively low temperatures. Spray drying yields concentrated and dry milk ingredients with excellent solubility, flavor, and color.

The spray drying process is typically a two-stage process that involves the spray dryer at the first stage with a static fluid bed integrated in the base of the drying chamber. The second stage is an external vibrating fluid bed. The product is moved through the two-stage process quickly to prevent overheating of the powder. The powder leaves the dryer and enters a system of cyclones that simultaneously cools it.

Roller drying is another process but is no longer widely used in the manufacture of most dry milk products. This process involves direct contact of a layer of concentrated milk with the hot surface of rotating rollers. It causes adverse effects of excessive heat on milk components. In this process, heat often causes irreversible changes such as lactose caramelization, Maillard reaction, and protein denaturation. Roller drying typically results in more scorched powder particles and poorer powder solubility than spray drying. However, roller dried milk absorbs more moisture than spray dried powder and is preferred in some food applications such as bakery products.

Instant NFDM is a processed NFDM to improve its dispersion properties. It reconstitutes readily in cold water. The instantizing process involves agglomeration, a process of increasing the amount of air incorporated between powder particles. In one process, a small amount of moisture is incorporated in dry milk particles suspended in air, forming porous aggregates, followed by re-drying and grinding the agglomerated particles. The process results in dry milk with improved reconstitution properties. During reconstitution, the air is replaced by water and incorporated air enables a larger amount of water to come into contact immediately with the powder particles.

Dry whole milk is the product resulting from the removal of water from milk, and it contains not less than 26% nor more than 40% milk fat and not more than 5% moisture (as determined by weight of moisture on a milk solids-not-fat basis). It is manufactured by spray drying whole milk with an added wetting agent, soy lecithin. Reconstituted extra grade whole milk powder possesses a sweet, pleasant flavor. It may have a slight degree of feed flavor, a definite degree of cooked flavor, and no off-flavors. The product should be free of graininess on reconstitution and exhibit no burnt particles. Dry whole milk is used primarily in confectionary, dairy and bakery products.

Dry buttermilk results from the removal of water from liquid buttermilk derived from the churning of butter. It should not be confused with the cultured product known as cultured buttermilk. It contains not less than 4.5% milk fat and not more than 5% moisture. The protein content of dry buttermilk is not less than 30%. Dry buttermilk is used in dairy foods such as ice cream and in other foods such as bakery items, dry mixes, and confectionary.

Dry buttermilk contains higher milk fat than NFDM. It contains a significant level of phospholipids, which act as emulsifying agents. The shelf life due to phospholipids is considerably reduced because they are prone to degradation, causing fishy odors and flavor defects.

Dry buttermilk product is another form of dry buttermilk. This designation indicates that it does not meet the specification of 30% minimum protein content. This product specifies protein content on the label. Except for protein content, dry buttermilk product meets all other standards of dry buttermilk.

Dry buttermilk product results from the removal of water from liquid buttermilk derived from the churning of butter. It does contain not less than 4.5% milk fat and not

more than 5% moisture. Dry buttermilk product contains less than 30% protein; its label should specify the minimum protein content.

Cultured/Fermented Dairy Products

Fermentation not only conserves vital nutrients of milk but also modifies certain milk constituents to enhance their functional and nutritional status. Culturing generates live and active cultures in significant numbers to provide distinct heath benefits beyond conventional nutrition to the consumer. For more information on cultured milks and yogurt, see the publications of Vedamuthu (2006) and Vasiljevic and Shah (2008). Chapter 13 of this book deals with this subject. Chapter 14 of this book contains information on functional bio-ingredients derived from dairy fermentations. Figure 1.6 shows an outline for the manufacture of cultured/fermented milks including yogurt, cultured buttermilk, sour cream, cream cheese, and cottage cheese.

Yogurt

Yogurt is a semisolid fermented product made from a heat-treated and standardized milk mix by the activity of a symbiotic blend of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Prebiotic and probiotic cultures currently are also used

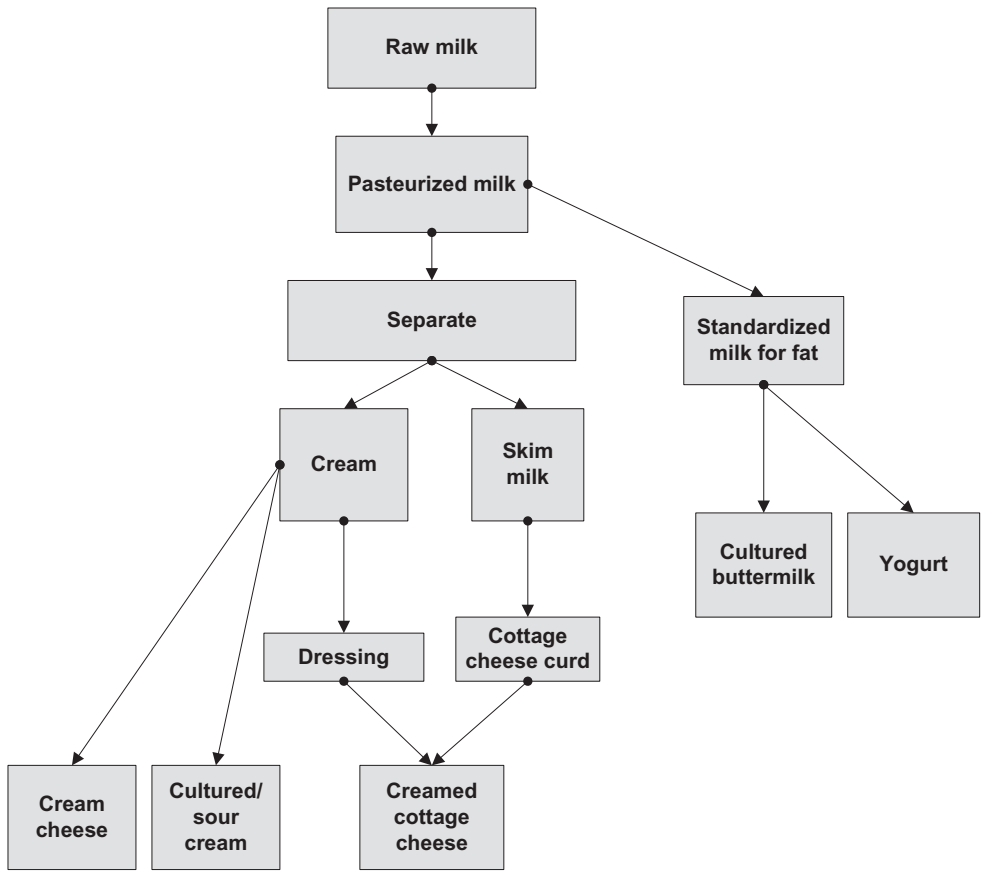

Figure 1.6. Processes for cultured dairy products.

Table 1.8. Typical composition of some cheeses used as ingredients.

Product	% Water	% Fat	% Protein	% Lactose	% Ash
Cottage cheese curd	79.8	0.4	17.3	1.8	0.7
Cottage cheese, creamed	79.0	4.4	12.5	2.7	1.4
Cream cheese	53.7	34.9	7.5	2.7	1.2
Neufchatel	62.2	23.4	10.0	2.9	1.5
Ricotta	71.7	13.0	11.3	3.0	1.0
Cheddar	36.8	32.0	26.0	1.0	5.0
Mozzarella	54.1	21.6	19.4	2.2	2.6

Adapted from Chandan (1997)

in the manufacture of fermented milks and yogurt products to enhance the functional or wellness attributes. A detailed discussion on types of yogurt and their manufacture is available in Chandan and O'Rell (2006a and b). Plain yogurt is a raw material for frozen yogurt, certain margarine products, and salad dressings.

Cultured Buttermilk

Cultured buttermilk is obtained from pasteurized skim or part-skim milk cultured with lactococci and aroma-producing bacteria, leuconostocs (White, 2006). The product is bottled in paper/plastic containers. Cultured buttermilk creates desirable characteristics of texture to bakery items such as pancakes.

Sour/Cultured Cream

Sour/cultured cream is manufactured by culturing pasteurized cream with lactococci and aroma-producing bacteria, leuconostocs, and has a butter-like aroma and flavor (Born, 2006). Crème fraiche resembles sour cream, except it contains up to 50% fat as compared to 18% fat in sour cream and has a higher pH of 6.2 to 6.3. Cultured cream is used in making dips and is an integral constituent of Mexican cuisine.

Cheese

Cheese and cheese products are consumed as such or are used as ingredients in entrees, side dishes, and ready-to-eat snacks. These products are designed to be consumed as a spread, as slices in sandwiches, and dips or toppings on vegetables and grain snacks. For a more detailed discussion on cheese, see other publications (Chandan, 2003; Chandan, 2007c; Singh and Cadwallader, 2008). Chapters 10 and 11 of this book discuss natural and process cheese products in detail. Table 1.8 shows the typical composition of some cheese varieties used as ingredients in food processing.

Unripened Natural Cheese

Cottage Cheese

Cottage cheese belongs to the class of natural, unripened soft cheeses (Chandan, 2003). It is a relatively low-fat product with high protein content and is part of low-calorie diets, including salads. It is used as a filling in the preparation of the breakfast food blintze. Good-quality cottage cheese has a clean, creamy, cultured milk flavor, a natural creamy color, and a meaty, soft texture. It comes in small or large curd sizes. The curd is coated with a cream dressing.

Cream Cheese

Cream cheese contains at least 33% fat and not more than 55% moisture. It is a soft, unripened lactic-acid-coagulated cheese. It is made by culturing cream by a process similar to that of cottage cheese (Chandan, 2003). It has a mild, acid, and creamy flavor. It is a major ingredient of cheese cakes.

Ricotta Cheese

Ricotta cheese is made from whey or a blend of whey and milk by direct acidification with a food-grade acid. Ricotta made from 95% sweet whey and 5% milk contains 68% to 73% moisture, 16% protein, 4% to 10% fat, and 4% lactose (Chandan, 2003). Ricotta has a bland to a slightly cooked flavor. Its texture is soft and creamy. It is used in Italian cuisine, particularly in lasagna and ravioli.

Ripened Natural Cheese

Ripened natural cheese is made directly from milk and some cases cheese whey. It is made by coagulating or curdling milk, stirring and heating the curd, draining off the whey, and collecting or pressing the curd. Desirable flavors and textures are obtained in many cheeses by a curing process at a specified temperature, humidity, and time period. Many cheese varieties are used as ingredients in popular main-meal items. Functional cheese derivatives have been developed for use in specialty food products. For example, quick-melting cheese slices for cheeseburgers, cheese sauces for Mexican dishes, and high-melt cheese products for filling meat products are commercially manufactured to meet demands of fast food and other food service businesses.

Production of cheese ingredients is based on natural cheese as an intermediate ingredient. The principles of production of cheddar and other cheese varieties are discussed elsewhere (Singh and Cadwallader, 2008). Nevertheless, a summary of the procedure for cheese manufacture is given below.

Raw milk is standardized to a casein : fat ratio of 0.7, pasteurized, and transferred to a cheese vat at 31.1°C (88°F). Cheese color may be added as an optional ingredient. A cheese culture at 1% level is then mixed with milk. As the culture grows, acidity starts to build in the milk. When acidity rises by 0.05% to 0.1%, rennet (a coagulating enzyme) is added at the rate of 3 oz/1,000 lbs milk. The milk sets to a firm gel. After about 20 minutes, the gel is cut into one-quarter-inch or three-eighths-inch cubes by special wire-mesh knives. At this point, whey acidity should be 0.1% to 0.12% and the curd cubes and whey start to separate out. The next step is cooking, which involves raising the temperature of the vat contents from 31.1°C to 36.7°C to 38.9°C (88°F to 98°F to 102°F) until an acidity of 0.17% to 0.20% is obtained. Whey, the liquid portion, is drained, and the solid portion (curd) is allowed to build higher acidity at 37.8°C to 38.9°C (100°F to 102°F). Curd starts to knit or mat as a slab and the process of cheddaring is terminated at an acidity of 0.6%. The matted slab is then milled to form small size cheese curd, salted, and pressed into blocks or barrels. The blocks are then packaged and ripened at 7.2°C (45°F) for a period varying from 3 months to 1 year.

For retail sale, ripened cheese is packaged after cutting the blocks into 8-oz to 2-lb portions. Special wrapping materials are available to exclude entry of oxygen into the package and prevent loss of moisture. Plastic film pouches are formed to insert the cheese cuts, followed by evacuation of air and heat sealing. Shrinking of the wraps takes place by passing cheese packages through hot air/steam chambers to provide a skin-tight attractive appearance.

The whey fraction is separated to remove cream, condensed, and spray dried to produce sweet whey powder, which is widely used in several bakery items. Several whey fractions are clinically proven to possess bioactive properties and are now key constituents of functional or wellness foods.

Process Cheese and Products

Process Cheese

Natural cheese constitutes a main ingredient for the manufacture of process cheese and its products. Process cheese delivers fairly uniform flavor and texture as compared to

natural cheese. Its melting characteristics can be manipulated by use of specific melting (or emulsifying) salts. Normal variations in flavor of natural cheese are minimized by blending of mild and strong flavors (and ages) of natural cheese. Selected cheeses are macerated and transferred to a cooking vat. Emulsifying salts are used to prevent separation of fat during heat processing. The salts commonly used (up to 4% level) are citrates and phosphates. The salts result in desirable body of the product. If desired, other ingredients such as sodium chloride, preservatives, cream, dry milk, and whey may be added.

The mixture is heated to 79.4°C to 82.2°C (175°F to 180°F) for one to five minutes with vigorous agitation. Scraped surface equipment is necessary to facilitate heat transfer. The mixture turns fluid and a homogeneous mass is obtained. The product is ready for packaging into forms and cooled to obtain process cheese loaves. Process cheese contains higher moisture than natural cheese. To obtain cheese slices, molten cheese is subjected to casting on a roller drum, followed by cutting into ribbons and slices, and packaging.

Pasteurized Process Cheese

Pasteurized process cheese is the food prepared by comminuting and mixing, with the aid of heat and one or more cheese of the same variety or two or more varieties (except cream cheese, Neufchatel cheese, cottage cheese, creamed cottage cheese, cook cheese, hard grating cheese, semi-soft part-skim cheese, part-skim spice cheese, and skim milk cheese for manufacturing) with an emulsifying agent into a plastic homogeneous mass. Heating is at not less than 65.5°C (150°F) and for not less than 30 seconds. The moisture content is required to not exceed 1% more than the constituent natural cheeses, but cannot exceed 43% with a few exceptions. Process cheese is a pasteurized blend of American cheeses of different ages that comes in different flavors. American process cheese has mild cheddar flavor. Sharp American has sharp or aged cheddar flavor. American Swiss has mild Swiss flavor. The consistency of process cheese is relatively semi-firm, creamy, and smooth, as compared to natural cheese counterparts. Its functionalities are sliceability, extra-melt (melting easily on heating, does not thicken, and can withstand high temperature hold for long periods), and slow melt (maintains shape at high temperature). It may be flavored with seasonings. Process cheese is marketed as cheese loaf and slices.

Pasteurized Process Cheese Food

Pasteurized process cheese food is similar to pasteurized process cheese, except it must contain moisture not exceeding 44%, and fat content is not less than 23%. It contains optional dairy ingredients: cream, milk, skim milk, buttermilk, cheese whey solids, anhydrous milk fat, and skim milk cheese for manufacturing. The pH is adjusted to not below 5.0 with vinegar, lactic acid, citric acid, phosphoric acid, or acetic acid. It cannot contain more than 3% emulsifying agents. Sorbic acid (up to 0.2%) is allowed as a preservative. The product is obtained by blending American cheeses of different ages with nonfat dry milk and whey and other permissible ingredients, followed by pasteurization. It melts quickly to give a smooth liquid. Cold product can be sliced easily. Major uses include entrees, au gratin potatoes, sandwiches, and Mexican dishes. It may be flavored with seasonings, smoke, pimento, jalapeno, salami, pepperoni, etc. Moisture content is 44% maximum and fat in dry matter is 41% minimum.

Pasteurized Process Cheese Spread

Pasteurized process cheese spread contains even higher moisture and lower fat than process cheese food. It is more spreadable

than cheese food. It may contain meat, vegetables, pimento, or pineapple, or may be flavored with blue cheese, onion, etc. Its uses include snacks, deviled eggs, noodle casserole, meatballs, hot vegetables, sandwiches, sauces and dressings.

It is similar to process cheese food, but is spreadable at 21°C (69.8°F). It has a moisture content of 44% to 60% and its fat content is not less than 20%. It may contain optional dairy ingredients, emulsifying agents, and gums (less than 0.5%). Acids may be added to get pH to not less than 4.0. Sweetening agents may be used (sugar, dextrose, corn sugars). Sorbic acid (0.2% maximum) may be used a preservative.

Cold Pack Cheese (Club Cheese)

Cold pack cheese, or club cheese, is a cold blend of American cheese or Swiss cheese and may be smoke flavored. It spreads easily. It is used as an appetizer, snack, or dessert. The product involves blending without heating various cheeses. Only cheese from pasteurized milk is used. Its moisture content is the same as that of the individual cheese; the fat content in dry matter is not less than 47% in most cheese except Swiss (not less than 43%) and gruyere (not less than 45%). Cold pack cheese may contain acids to standardize pH to not below 4.5. Sorbic acid (less than 0.3%) can be used as preservative.

Cheese Powders

Spray dried cheese powders are widely used as seasonings and flavorings in grain-based snacks. They are produced by macerating cheese, dispersing in water at 35% to 40% solids concentration, adding emulsifying salts, homogenizing, and spray drying. Foam spray drying is considered to give a superior flavored product with larger particle size. In addition to cheese, dry milk, whey, vegetable oils, salt, enzyme-modified cheese concentrate, color, and seasonings may also be incorporated in the ingredient.

Cheese powders can be packed in nitrogen atmosphere to give a longer storage life. Hard Italian cheese (namely, parmesan) is dried after grating in tray or belt dryers in which dry, hot air is circulated to reduce moisture to less than 6%. The cheese is ground and packaged after cooling. Cheese powders are popular toppings in Italian cuisine. More details on cheese powders, enzyme-modified cheese, and cheese sauces can be found in Chapter 12.

Enzyme-modified Cheeses

Enzyme-modified cheeses (EMC) are cheese flavor concentrates obtained by treating raw cheese curd with specific lipases/pregastric esterases and proteases followed by fermentation with a cheese culture. It takes one to three days to develop a flavor concentration of 10- to 20-fold as compared to ripened cheeses. Cheese paste is then heat treated to stop the biochemical reaction, and cooled. The EMC may be purchased as a paste or it may be blended with whey and dried as a spray-dried powder. EMCs offer significant savings as substitutes for aged cheese in cheese-flavored crackers and fillings for bakery items. It also is an economical ingredient in process cheese manufacture.

Cheese Sauces

Cheese sauces are aseptically processed slurries that are canned for convenient use as dips or as sauces on nachos, potatoes, and pasta. Typically, the ingredients used are cheddar cheese, skim milk, whey, buttermilk, vegetable oil, starch, sodium phosphate, salt, caseinate, citrate, color, lactic acid, stabilizers, emulsifiers, and seasonings.

Whey Products

Whey, the greenish-yellow liquid produced from the manufacture of cheese, contains about half of the solids of whole milk. Its composition depends largely on the variety

of cheese being made. These solids are valuable additions to the functional properties of various foods, as well as a source of valuable nutrients. The techniques of concentration, drying, and reverse osmosis recover virtually all of the whey solids. Crystallization, ion exchange, and membrane systems such as ultrafiltration and electrodialysis are used for fractionating whey into concentrates of protein, minerals, and lactose. See Kilara (2008) and Chapter 8 of this book for details. Figure 1.7 shows an outline for the manufacture of whey products and milk protein concentrate. The proximate composition of dry whey, whey products, caseinates, and milk protein concentrates is shown in Table 1.9.

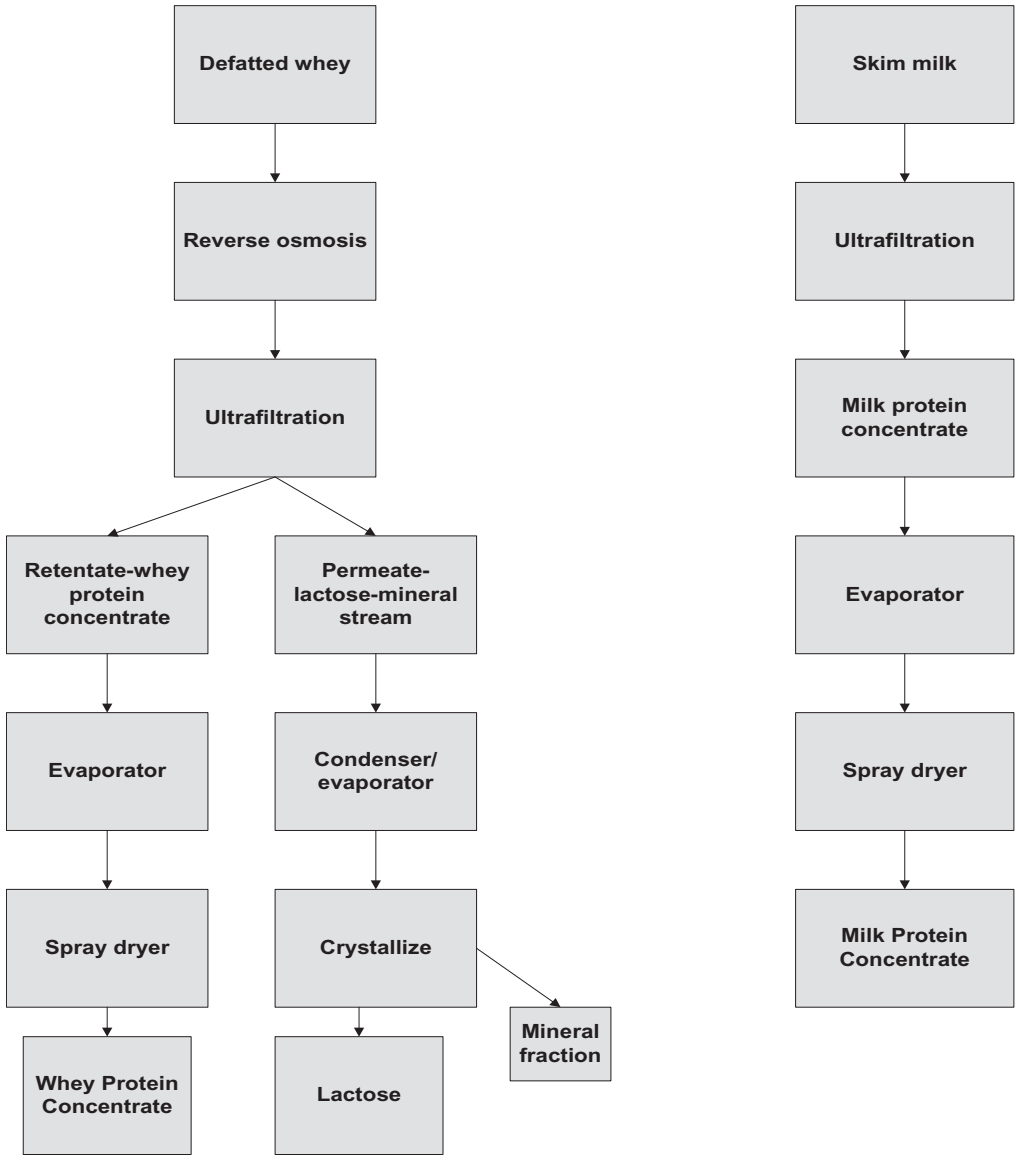

Figure 1.7. Process outlines for whey products and milk protein concentrate.

Table 1.9. Proximate composition of dry whey and other dairy products.

Product	% Water	% Fat	% Protein	% Lactose	% Ash
Dry sweet whey	4.5	1.1	12.9	73.5	8.0
Reduced lactose whey	4.0	2.5	22.0	55.0	16.5
Demineralized whey	4.0	2.2	13.0	76.8	4.0
Dry acid whey	4.3	1.0	12.3	71.3	11.1
Whey protein concentrate, 34% protein	3.5	4.0	34.5	51.0	7.0
Whey protein concentrate, 50% protein	3.5	4.0	50.5	36.0	6.0
Whey protein concentrate, 80% protein	3.5	6.0	80.5	5.0	5.0
Whey protein isolate	3.5	0.5	93.0	1.0	2.0
Acid casein	9.0	1.0	88.0	0.1	1.9
Rennet casein	11.0	1.0	85.0	0.1	2.9
Calcium caseinate	3.5	1.0	91.0	0.1	4.4
Sodium caseinate	3.0	1.5	90.9	0.1	4.5
Milk protein concentrate	4.0	3.0	65.0	22.0	6.0
Food grade lactose	0.5	0.1	0.1	99.1	0.2
Dairy minerals concentrate	10.0	1.0	8.0	1.0	80.0

Adapted from Chandan (1997), Sodini and Tong (2006), Chandan and O'Rell (2006a)

Dry Sweet Whey

Dry sweet whey is produced by drying defatted fresh whey obtained from the manufacture of cheddar, Swiss, and other cheeses. It contains all the constituents except water in the same relative proportion as in liquid whey. Dry acid whey is similar to dry sweet whey but is produced by drying fresh whey obtained from cottage and ricotta cheese manufacture.

Spray drying condensed whey converts sweet whey into a stable, nonhygroscopic, and noncaking product. In this process, high-solids whey concentrate is spray dried to a free moisture content of 12% to 14%, causing lactose to take on a molecule of water and become crystallized. This causes whey solids to convert from a sticky, syrupy-like material into a damp powder with good flow characteristics. For drying acid cottage cheese whey, a commercial dryer combines spray drying with through-flow continuous bed drying. The concentrate is spray dried in the hot air chamber to 12% to 15% moisture. The particles fall to a continuous, porous, stainless-steel belt where lactose undergoes rapid crystallization. Crystallization of lactose before final drying is necessary for drying acid whey. A belt conveys the product to another chamber where the whey is further dried by dehumidified air that moves through the porous bed.

Dry sweet whey is widely used in bakery products, dry mixes, process cheese foods and spreads, frozen desserts, sauces, meat emulsions, confections, soups, gravies, snack foods, and beverages. Dry acid whey has an additional functional attribute of providing acid flavor in certain foods and it imparts desirable textural properties to bakery items.

Fractionated Whey Products

Membrane technology is used for partial concentration (reverse osmosis); fractionation of solutes (lactose, minerals) from macromolecules such as proteins, fat globules, colloidal particles, (ultra-filtration); and demineralization (ion-exchange, electro-dialysis) of whey, its fractions, and milk. These processes produce highly functional ingredients. The membrane processes are pressure-activated processes that separate components on the basis of molecular size and shape. Reverse osmosis is the process in which virtually all species except water are rejected by the membrane. The osmotic pressure of the feed stream in such a system is often quite high. Consequently, to achieve adequate water flux rates through the membrane, such systems often use hydrostatic operating pressures of

5,883.6 Kg/cm^2 (600 psi) or greater. Ultrafiltration refers to the process in which the membrane is permeable to relatively low-molecular-weight solutes and solvent (permeate), but is impermeable to higher molecular weight materials (retentate). The permeability and selectivity characteristics of these membranes can be controlled during the fabrication process so that they retain only molecules above a certain molecular weight. Thus, ultrafiltration is essentially a fractionating process, whereas reverse osmosis is effectively a concentrating process.

One advantage of ultrafiltration over other processes is that by varying the amounts of permeate removed, a wide variety of protein concentrates, ranging up to 60% protein, can be obtained. Higher levels can be obtained by simultaneously adding fresh water and further concentrating by ultrafiltration by a process called diafiltration.

The permeate is used for manufacture of milk sugar, lactose, by condensing and crystallization. Lactose crystals are harvested and dried in a tumble dryer.

Reduced Lactose Whey

Reduced lactose whey is produced from whey by partial crystallizing out lactose and recovery of mother liquor by centrifugation, followed by drying. Lactose content of the dry product is 60% or less.

Reduced Minerals Whey

Reduced minerals whey is produced from whey by selective removal of a portion of minerals. Ash content of the dry product is 7% or less. Demineralization processes have helped in the development in an array of whey products. Excessive mineral content makes dry whey distasteful, and can have an adverse affect on the physical properties of some foods. The two most widely used demineralization processes for whey are ion exchange and electrodialysis.

In the ion-exchange process, whey is passed through two containers that are filled with special synthetic resins that have the ability to exchange ions. In the first container, the special synthetic resins exchange hydrogen ions for cations in the whey. Here the positive ions of the salt are captured and acid is formed by the release of hydrogen ions. The whey is then passed over the anion exchanger where hydroxyl ions are exchanged for negative ions of the salt, and water is formed. When the mobile ions of the resins are completely replaced by other ions, the resin must be regenerated for further use.

Electrodialysis, a combination of electrolysis and dialysis, is the separation of electrolytes under the influence of an electric potential through semi-permeable membranes. The driving force is an electric field between the anode (positively charged) and the cathode (negatively charged). Between the anode and the cathode, a number of ion-selective membranes are placed which are permeable only to anions or cations. Every other membrane has a positive charge repelling positive ions and allowing negative ions to pass, and in between there is a negatively charged membrane doing just the opposite.

In principle, whey is pumped through every second space between two membranes, and a solution of sodium chloride (cleaning solution) is pumped through the compartments between the whey streams. The ions move from the whey stream into the cleaning solution where they are retained, because they cannot move any farther. The cleaning solution contains minerals, acid, some lactose, and small nitrogenous molecules. The membranes are cleaned chemically. Protein molecules remain in the fluid while the minerals are removed. The process results in a protein concentrate.

Lactose

Lactose is crystallized from condensed whey or from permeate (50% to 60% solids)

obtained by ultrafiltration fractionation of milk or whey. The supersaturated solution is cooled under specific conditions to crystallize lactose. Lactose crystals are harvested and washed to remove the mother liquor and dried. Crude lactose obtained this way contains approximately 98% lactose. Edible and USP grades are produced from crude lactose by protein precipitation, de-colorization with activated carbon, and subsequent demineralization. Lactose is further refined by recrystallization, followed by spray drying.

Whey Protein Concentrates and Isolates

Whey protein concentrates are products derived from whey by removal of minerals and lactose. The process of protein concentration uses ultrafiltration, electrodialysis, and ion exchange technologies. On dry basis, the protein concentrate contains a minimum of 25% protein. Whey protein isolate contains at least 90% protein.

Whey protein concentrate of 34% protein is commonly used in yogurt, bakery mixes, dietetic foods, infant foods, and confections. Its water binding, fat-like mouth feel, and gelation properties are particularly useful in these products. Whey protein concentrate of 50% or 80% protein offers distinct functional attributes. It is especially suited for use in nutritional drinks, bars, soups, bakery items, meat products, dietary foods, and protein-fortified beverages. It gives clear suspensions over a wide pH range and has a bland flavor. Some applications require undenatured ingredients to maximize water-binding capacity during food processing. It is also available in a gel-forming version. Fractionated and hydrolyzed whey protein products are now marketed as health-promoting functional foods.

Casein and Caseinates

Casein is obtained from pasteurized skim milk by precipitation with an acid, followed by drying. This gives acid casein. Acid casein is produced by precipitation of skim milk with hydrochloric acid, sulfuric acid, acetic acid, or lactic acid at pH 4.6. Casein derived from the action of rennet (chymosin) is called rennet casein. Micellar casein is also commercially available. They all have distinctive functional characteristics.

Caseinates are derived from casein by treatment with a suitable alkali. Casein is basically insoluble in water, whereas caseinates are easily dispersible. Acid casein is neutralized to pH 6.7 with sodium hydroxide for the production of sodium caseinate. Similarly, potassium hydroxide and calcium hydroxide yield potassium and calcium caseinates, respectively. This subject is discussed in Chapter 7.

Milk Protein Concentrate

Milk protein concentrate is obtained by ultrafiltration of skim milk and subsequent spray drying. The protein content varies according to the application in dairy products and functional foods. An outline for the manufacture of milk protein concentrate is shown in Figure 1.7.

The applications of dairy ingredients in dairy foods are discussed in Chapter 17. Chapter 18 gives the applications in bakery, meat products, dressings, sauces, and soups. Chocolates and confections are given in Chapter 19. Infant formulas, nutritional drinks, and bars are discussed in Chapter 20.

Trends in Availability and Use of Major Dairy Ingredients

Selection of a dairy ingredient is largely based on the desired contribution of certain milk constituents such as milk fat and solids-not-fat (proteins, lactose, and minerals) in a given food. Cost and availability also contribute significantly to the use of a particular ingredient. It is helpful to understand recent trends in production of major dairy products

Table 1.10. Production of major dairy ingredients in 2008.

Ingredient	Production, 1,000 pounds	% Change from 2007
Milk	189,992,000	2.3
Butter	1,644,078	7.3
Total cheese	9,934,530	1.6
Canned evaporated and condensed whole milk	587,745	18.2
Canned evaporated skim milk	18,313	−1.2
Bulk sweetened condensed whole milk	83,100	5.3
Bulk unsweetened condensed whole milk	134,824	27.2
Bulk sweetened condensed skim milk	29,106	NA
Bulk unsweetened condensed skim milk	1,509,246	−7.9
Condensed/evaporated buttermilk	64,115	15.0
Dry whole milk	50,137	57.9
Nonfat dry milk, human grade	1,519,173	17.0
Dry skim milk, animal grade	8,283	70.3
Dry skim milk protein standardized and blends	373,830	86.3
Dry buttermilk	72,494	−10.9
Sour cream	1,127,079	−0.7
Cream and Neufchatel cheese	763,692	−1.2
Condensed whey, sweet, human grade	103,936	NA
Dry whey, total	1,107,539	−2.3
Reduced lactose and mineral dry whey, human food grade	36,962	NA
Reduced lactose and mineral dry whey, animal grade	51,788	NA
Whey protein concentrate, 25.0% to 49.9% protein	286,214	NA
Whey protein concentrate, 50.0% to 89.9% protein	137,433	NA
Whey protein isolate, 90% or higher protein	43,204	NA
Lactose, human and animal grades	755,295	−1.4

NA, not available
Source: USDA (2009)

(Table 1.10) impacting their availability and cost.

In 2008, 14.7% of the U.S. milk supply was used as fluid milk and 7.3% for fluid cream products. The remainder was used in production of cheese (40.6%), butter (19%), frozen desserts (7.9%), dry milk products (0.5%), and cultured dairy products (4.9%). About 0.6% of milk production was used on the farms, and other uses accounted for 4.6% of milk supply (IDFA, 2009).

In 2002, uses of nonfat dry milk (in million pounds) were as follows:

- Dairy: 617.1
- Bakery: 59.6
- Confectionery: 58.2
- Pharmaceutical, special dietary, nutraceuticals: 58.2
- Prepared dry mixes: 52.5
- Beverages: 24.5
- Infant formula: 19.3
- Animal feed: 11.6
- Meat processing: 8.3
- Institutional use: 7.4
- Soups: 3.4
- Margarine: 1.2
- Packaged for retail: 1.2
- Other uses: 4.2

Dry whole milk was used (in million pounds) in confectionary (39.1), prepared dry mixes (3.6), dairy (3), packaged for retail use (2.1), bakery (1.1), animal feed (0.2), and institutional use (0.1). Dry buttermilk (a byproduct of butter manufacture) was used (in million pounds) as follows: 15.7 in prepared dry mixes, 15.1 in bakery, 9 in dairy, 3.6 in confectionery, 0.7 in animal feed, and 0.3 in other uses.

The cheese industry has been a growth industry for many years. A record high production 9.9 billion pounds was registered in 2008. Estimated total wholesale value of

Table 1.11. Per capita consumption (in pounds) of various natural and process cheese products in the United States in the years 2005 to 2008.

Product	2005 consumption, in pounds	2006 consumption, in pounds	2007 consumption, in pounds	2008 consumption, in pounds
Total natural cheeses	31.76	32.70	33.19	32.48
Cheddar cheese	10.10	10.37	10.03	9.96
Total American cheeses	12.90	13.10	12.80	13.00
Total Italian cheeses	13.30	13.80	14.30	14.10
Mozzarella	10.18	10.53	11.05	10.65
Ricotta	0.80	0.82	0.89	0.84
Provolone	1.03	1.08	1.09	1.09
Romano	0.22	0.25	0.28	0.32
Parmesan	0.58	0.64	0.64	0.78
Cream and Neufchatel	2.41	2.53	2.56	2.51
Swiss	1.27	1.27	1.28	1.20
Muenster	0.26	0.32	0.34	0.39
Blue	0.20	0.20	0.20	0.19
Brick	0.03	0.02	0.02	0.02
Hispanic	0.57	0.61	0.63	0.64
Processed cheese	4.16	4.06	4.11	4.09
Process food and spreads	3.47	3.72	3.38	2.97
Total processed cheese and products	7.63	7.79	7.49	7.06

Adapted from IDFA Dairy Facts (2009)

cheese and cheese products in 2007 was $33 billion.

Most of the growth in natural cheese category can be attributed to growth in mozzarella and pizza cheese. The U.S. production of Italian style cheese increased to 4,158 million pounds in 2008. Mozzarella cheese constitutes 78% 0f Italian cheese varieties. For comparison, production of American cheese varieties was a little lower than that of Italian cheeses and amounted to 4,071 million pounds.

The per capita consumption of natural and processed cheese products is given in Table 1.11.

The 2008 cheese consumption (excluding cottage cheese) reached a record level of 32.4 pounds per capita. Although there are more than 300 varieties of cheese available in the U.S., the most popular cheeses include Mozzarella with per capita consumption of 10.65 pounds, followed by cheddar at 9.96 pounds per capita. Consumption of all Italian cheeses and all natural cheeses continues to grow. Trends in total processed cheese consumption show a slight decrease.

Note: Some of the information in this chapter has been derived from Chapter 1 of Dairy Processing and Quality Assurance: An Overview, published in *Dairy Processing and Quality Assurance* (Wiley-Blackwell, 2008).

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Chapter 2

Chemical, Physical, and Functional Characteristics of Dairy Ingredients

Stephanie R. Pritchard and Kasipathy Kailasapathy

Milk

Milk is a white, lacteal secretion of the mammary gland that provides neonates with their required nutritional needs. It can be obtained from mammals including humans, cows, buffaloes, goats, and sheep. Cow's milk is the main milk consumed by humans and it is defined as a whole, clean, lacteal secretion obtained by complete milking of one or more healthy milk animals, excluding that obtained within 15 days before parturition and 15 days after calving or such periods as may be necessary to render the milk practically colostrum-free and containing the minimum-prescribed percentages of milk fat and milk-solids-non-fat (SNF).

Chemically, it consists of multidisperse phases that contain significant levels of required nutrients including fat, protein, carbohydrates, vitamins, and minerals.

Dairy Ingredients

Milk contains a number of ingredients that are used in various food products due to their characteristic properties that include the emulsifying and stabilizing properties of caseinates, the gelling properties of whey protein concentrates and isolates, and the use of lactose in confectionary products.

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Milk Components and Other Definitions

Several components of milk can be segregated using various techniques and consequently are named accordingly. Milk serum is milk without the fat globules and casein micelles. Milk plasma is the fluid portion of the milk minus the fat globules. The total content (except water) is referred to as dry matter. The milk-solids-not-fat content consists of protein, lactose, and minerals. Total solids include the serum solids and milk fat.

Chemical Properties of Milk

Milk constituents (protein, lipids, etc.) have various chemical and physicochemical properties that affect their processing and functional characteristics, which are significant in the production of milk-derived dairy products including cheese, yogurt, butter, and cream.

Milk Composition

Milk is mainly comprised of water, followed by lactose, fat, protein, and minerals.

Constituents of Milk

The constituents of milk are the same for all species; however, the concentration of each constituent varies. Legally, milk refers to cow's milk. Milk from other species must be labeled accordingly. Table 2.1 shows the

Table 2.1. Typical chemical compositions of milk from various mammalian species (g/100 g).

Species	Water	Fat	Protein	Lactose	Ash
Donkey	90.0	1.3	1.7	6.5	0.5
Buffalo	84.2	6.6	3.2	5.2	0.8
Camel	86.5	3.1	4.0	5.6	0.6
Cow	86.6	4.6	3.4	4.9	0.5
Sheep	79.4	8.6	6.7	4.3	1.0
Goat	86.5	4.5	3.5	4.7	0.8
Human	87.7	3.6	1.8	6.8	0.1
Horse	89.1	1.6	2.7	6.1	0.5
Yak	82.7	6.5	5.8	4.6	0.8

Adapted from Kailasapathy (2008) and Francis (2000)

typical chemical compositions of milk from different mammalian species.

Water

Water is the principal constituent in milk, totaling 79% to 90%, depending on the species. It encompasses all other constituents of milk (total solids) that are either dissolved or suspended in it. In addition, small amounts of water are hydrated or bound chemically to lactose, salt, or protein.

Regulations prohibit the addition of water to raw milk. The water activity in milk is relatively high, 0.993. The removal of water from milk results in an increased shelf life, and this property has been exploited through the production of powdered milk, from which the water is removed.

Milk Fat

Milk fat is mostly contained in fat globules that are protected by a membrane. The concentration and composition of milk lipids depend on several factors including breed, species, feed, individuality, lactation stage, milking interval and stage, and presence of mastitic infection.

The fat globules range from 1 to 20 µm in diameter. They are made up of approximately 98% triglycerides, 0.2% to 1% phospholipids, and 0.2% to 0.4% sterols. The phospholipids mostly associate with the fat globule membrane. They also contain traces of fatty acids; vitamins A, D, E, and K; and enzymes. More than 400 different fatty acids have been identified in milk. The predominant fatty acids in bovine milk are myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), and oleic acid ($C_{18:1}$) (Otter, 2003).

The fat globule membrane is comprised mainly of phospholipids and proteins, as well as lipids, lipoproteins, cerebrosides, nucleic acids, enzymes, trace elements (minerals), and some bound water molecules that stabilise and prevent the fat globules from coalescence during milk processing and handling. The fat globule membrane prevents attack from lipases, which would otherwise break down the lipids (lipolysis) into short fatty acids, therefore increasing the amount of diglycerides, monoglycerides, and free fatty acids in milk. The free fatty acids are fairly water-soluble and are situated in milk plasma and fat. Short free fatty acids situated in the milk plasma are ionized and more water-soluble than long free fatty acids ($>C_{14}$) found in fat and at the oil-water interface.

Several types of minerals are associated with the fat globule membrane including copper and iron in relatively large amounts, 5% to 25% and 30% to 60%, respectively. Other minerals include cobalt, calcium, sodium, potassium, magnesium, manganese, molybdenum, and zinc.

Compound lipids also occur in milk such as phospholipids and phosphatides that are situated mainly in the fat globule membranes but also in the milk plasma, lipoproteins, and milk microsomes. The total phospholipid content in milk is approximately 36 mg/100 ml. Phospholipids and phosphatides are highly surface active and polar, and dissolve poorly in both water and oil.

Lipids can be crystallized, which affects the fat structure, melting range, and rheological properties of milk. Furthermore, autoxidation of the double fatty acid bonds or residues can occur, leading to off flavors.

Whole milk contains 10 to 20 mg/100 g cholesterol (3.3% fat). The amount of cholesterol is positively correlated with the amount of fat in the product. Cholesterol is located in the fat globule membrane, and approximately 10% of the cholesterol is esterified. The amount of cholesterol in milk is affected by various factors including species, breed, feed, season, and stage of lactation; the amount of cholesterol is highest near the end of lactation. The average cholesterol content of various dairy products and their corresponding fat percentages are shown in Table 2.2.

Table 2.2. Average cholesterol and fat content of various dairy products.

Product	% Fat	Cholesterol (mg/100 g)
Whole milk	3.3	14
Skim milk	0.25	2
Goat milk	3.92	11
Human milk	4.03	25
Buttermilk	0.51	1.4
Yogurt	3.75	10
Cream	34.87	110
Whipped cream	31.7	84
Sour cream	18	48
Ice cream	10.77	45
Blue cheese	28.74	75
Cottage cheese (creamed)	4.51	15
Swiss cheese	27.45	92
Cheddar cheese	33.14	105
Butter	81.11	219

Adapted from Kailasapathy (2008) and Souci (2008)

Milk Proteins

Proteins are made up of amino acids with specific properties that are determined by the side chains of the amino acids in the polypeptide chain. The conformation of the protein depends on the hydrogen bonds, hydrophobic interactions, and salt bridges formed between the peptide chains. Regular arrangements include β-sheets and α-helices. Temperature, ionic strength, and pH affect protein conformation.

The major classes of protein in milk are casein and whey or serum proteins. Casein makes up approximately 80% of the milk protein, and whey or serum proteins make up approximately 20%. The proteins are synthesized in the mammary gland, and therefore, are derived genetically. The protein content of milk remains constant with a concentration range of 30 to 35 g/kg. However, it is influenced by the lactation stage of the cow.

Table 2.3 shows the typical protein composition of bovine milk.

Casein Proteins

Four main types of casein have genetic variants: α_{s1}-casein, α_{S2}-casein, β-casein, and κ-casein. They are phosphorylated and hydrophobic and associate with themselves and each other. They represent 38%, 10%,

Table 2.3. Typical protein composition of bovine milk.

Protein Component	Weight (g/kg)
Total protein (%)	35.1
Total casein	28.6
Whey protein	6.1
α_{s1}-casein	11.5
α_{s2}-casein	3.0
β-casein	9.5
κ-casein	3.4
γ-casein	1.2
α-lactoglobulin	1.2
β-lactoalbumin	3.1
Serum albumin	0.4
Immunoglobulin	0.8
Proteose peptone	1.0

Adapted from Kailasapathy (2008)

36%, and 13% of whole casein, respectively. Overall, 21 variants of casein have been identified that occur by genetic mutations such as the deletion or the substitution of amino acids.

Caseins have distinct disordered molecular structures that lack disulfide bridges, and therefore they are very heat stable, withstanding temperatures above 140°C before dissociating. Their hydrophobicity is due to the high ratios of apolar amino acids including valine, leucine, isoleucine, phenylalanine, and proline (between 35% and 45%). However, this is counteracted by the high phosphate content and low concentrations of sulphur-containing amino acids such as methionine and cysteine that allow the caseins to be reasonably water soluble. Furthermore, their susceptibility to proteolysis is due to the lack of secondary and tertiary structures, particularly the proteins α_{s1}-casein and β-casein.

κ-casein consists of 169 amino acids and is approximately 19 kDa, and it contains both glycosylated and phosphorylated residues. It can exist as a dimer up to a decamer with the subunits held together by disulfide linkages. Unlike the other caseins, it is not sensitive to calcium and surrounds the micelles, keeping them intact. It usually contains one phosphoserine unit; however, genetic variants containing two or three phosphoserine units have been identified. Further, nine variants have been identified that demonstrate different degrees of glycosylation.

α_{s1}-casein consists of 199 amino acids and is approximately 23.6 kDa. It has the highest charge of all the casein molecules. It consists of at least eight phosphoserine units; however, the genetic variant α_{s0}-casein contains nine. It has 17 proline residues that ultimately disrupt the formation of secondary structures, such as α-helices and β-sheets.

α_{S2}-casein consists of 207 amino acids and is approximately 25.4 kDa. It is the least hydrophobic casein molecule. There are several genetic variants that contain between 10 and 13 phosphoserine units. Unlike β-casein and α_{s1}-casein, which contain no cysteine residues, α_{S2}-casein contains two cysteine residues. It exists as a dimer in milk.

β-casein consists of 209 amino acids and is approximately 24 kDa. It is the most hydrophobic casein molecule. There are six known genetic variants that contain between zero and five phosphoserine units. Similar to α_{s1}-casein, β-casein has few secondary structures due to the presence of 35 proline residues.

γ-casein is derived by hydrolysis of β-casein by the enzyme plasmin. Three variants have been identified near the C-terminal end of the β-casein molecule: which are γ^1 f(29–209), γ^2 f(106–209/11) and γ^3 f(108–209).

Casein micelles consist of a large portion (approximately 95%) of casein proteins that interact with each other and calcium. They vary in size from 80 to 1,000 nm with an average diameter of 150 nm in bovine milk. The micelle sizes of sheep and goat milk are different, and the caprine micelles are less heat stable than bovine milk micelles (Park et al., 2007).

Bovine casein micelles contain water, protein (about 94%), and salts (about 6%), including calcium, phosphorus, magnesium, citrate (these four ions are known as colloidal calcium phosphate [CCP]), and other traces of metals, enzymes (lipases, esterases, proteases), and milk serum.

Several models have been proposed for the structure of the casein micelles, including the coat-core model and sub-structure model, as well as the proposal that proteins exist as a porous network. The sub-structure model is the most evolved. It suggests that casein micelles contain submicelles that range from 12 to 15 nm in diameter and contain approximately 20 to 25 casein molecules and water (2 to 5 g/protein) with some submicelles containing K-casein.

Whey Proteins

Whey proteins are hydrophobic, globular, highly ordered proteins that contain disulfide linkages. Unlike casein proteins, whey pro-

teins have well-developed secondary, tertiary, and quaternary structures and poorer heat stability because they denature at temperatures greater than 75°C.

The two principal whey proteins in milk are α-lactalbumin and β-lactoglobulin, which are synthesized in the mammary gland. They constitute approximately 20% and 40% of total whey protein in bovine milk, respectively. Other whey proteins are proteose peptones, immunoglobulins, and serum albumin.

α-lactalbumin is a spherical, glycosylated, compactly folded calcium metalloprotein that consists of approximately 142 amino acids and is approximately 14 kDa. It is synthesized and secreted by the mammary gland, contains four disulfide bonds and eight cysteine residues, and is rich in tryptophan. Three genetic variants have been identified. It is the principal protein in human milk.

β-lactoglobulin consists of 178 amino acids with an approximate molecular weight of 18 kDa. It exists in both the monomeric and dimeric form at equilibrium in bovine milk; however, its association depends on temperature, pH, protein concentration, and ionic conditions. The hydrophobic dimeric form linked by one to three disulfide bonds is approximately 36 kDa. The genetic variants, A and B, are predominantly isolated from bovine milk; however, overall five genetic variants have been identified.

Higher concentrations of β-lactoglobulin are present in bovine milk when compared with human milk. Furthermore, it has better heat stability than α-lactalbumin due to the presence of one free sulphohydryl unit. It contains an open β-barrel enclosing a hydrophobic cleft and a single three-turn α-helix. It binds to several hydrophobic molecules including retinol and fatty acids via the hydrophobic cleft, which in turn stimulates lipase activity.

Immunoglobulins are antibodies that are synthesized in response to specific antigens. They are large, heterogeneous molecules found in the blood. The main immunoglobulins in milk are IgG, IgG2, IgA, and IgM. They provide offspring with protection against pathogenic microorganisms and their toxins, and the mammary gland against infection. Approximately 0.7 to 1 mg/ml is present in bovine milk. The basic structural unit of the immunoglobulins is similar, consisting of two heavy and two light chains joined together by disulfide bonds. The molecule is approximately 180 kDa in size.

Immunoglobulin G consists of one structural unit and is the main immunoglobulin in milk. Immunoglobulin M is a pentamer containing five structural units, which are linked by the junction component, and it has an approximate molecular weight of 900 kDa. Immunoglobulin A is a dimer made up of two structural units linked by the secretory component and junction component.

Bovine serum albumin consists of 582 amino acids and it is the longest protein. It is approximately 66 kDa and is predominantly composed of α-helices. It makes up approximately 1% to 5% of total whey protein. It is synthesized in the liver and enters the milk via secretory cells.

Proteose peptones are derived from the hydrolysis of β-casein. They are considered whey proteins because they elute in the whey fraction when isolated from milk. Three variants have been identified beta-casein f(1–105/7), f(1–28), and f(29–105/7). Proteose peptones are heat stable and acid-soluble proteins that are mainly responsible for the foaming of skim milk. They inhibit rancidity and have an immunological role (Fox, 2003).

Lactoferrin is a globular glycoprotein that is approximately 74 kDa and binds to iron (Fe) as it contains two metal binding sites. Bovine milk contains approximately 20 to 200 mg/L and human milk contains 2 g/L.

Several other minor whey proteins exist in milk including growth factors, vitamin-binding proteins (folate, vitamin D, riboflavin, and vitamin B_{12}), angiogenins, and osteopontin.

Non-protein nitrogen compounds including urea, uric acid, creatine, creatinine, and

hippuric acid are present in milk in trace amounts.

Carbohydrates

There are several carbohydrates in milk including lactose, glucose, galactose, and glycoconjugates (oligosaccharides, glycoproteins, and glycoaminoglycans). The main carbohydrate in milk is lactose, which ranges between 4% and 5% of total milk content, depending on the milk yield and lactation stage of the cow. The amount of lactose decreases as the lactation stage advances. It is a disaccharide comprised of α/β-D-glucose and β-D-galactose that are linked by a β1-4-O-glycosidic bond. Lactose exists in three forms: α-lactose monohydrate, β-lactose, and anhydrous α-lactose. The β-lactose form has the greatest solubility and is sweeter than the α-lactose forms. Lactose is the major food source for bacteria during the fermentation of milk. The bacteria hydrolyse the milk into glucose and galactose to produce lactic acid, which inhibits the growth of most other microorganisms.

Glucose, galactose, and oligosaccharides are present in relatively small concentrations, approximately 1 mg/ml.

Trace Elements

There are many trace elements in milk including minerals, salts, and vitamins. Their concentrations affect several physical properties including heat stability, electrical conductivity, the oxidation-reduction potential, and colligative properties.

Minerals and Salts

Milk contains all minerals considered essential for human nutrition including potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), chloride (Cl), and phosphate esters that make up between 0.7% and 0.8% of total milk content. Sodium, potassium, and chloride are present in milk as free ions that are readily diffusible. Their concentrations are negatively correlated with lactose.

The concentrations of calcium, magnesium, ionized phosphate, and citrate depend on the casein content in the milk. The concentration of citrate varies depending on season and diet of the cow; consequently, this may affect the soluble calcium content and milk stability, particularly the equilibrium between colloidal dispersion and salts. If the colloidal equilibrium is destabilized, the concentration of minerals in milk may affect the processing and require the addition of anions to bind to ionic calcium that would restabilize the caseins against aggregation. The concentration of calcium in milk is relatively high; therefore, milk is considered to be an important source of calcium.

Vitamins, Minor Components, and Micro-nutrients

Milk contains both fat-soluble and water-soluble vitamins. The concentration of each vitamin varies depending on the lactation stage, diet, and the health of the animal. The fat-soluble vitamins A, D, E, and K are situated within fat globules. Therefore, low-fat and skim milk contain smaller concentrations of fat-soluble vitamins; however, they are fortified to contain the same concentrations as whole milk. Vitamin A is important in vision and bone health. Vitamin D is important in bone health and immune system functions, and vitamin E (tocopherol) is an antioxidant. Vitamin K is present in milk; however, the amount contained is nutritionally insignificant.

The water-soluble vitamins B and C are present in milk. Milk contains adequate concentrations of B vitamins including thiamine, riboflavin, niacin, biotin, and folic acid. The concentration of vitamin C in milk is insignificant because it is destroyed by pasteurization. Table 2.4 shows the vitamin composition of various milks.

Table 2.4. Vitamin composition of various milks.

Vitamins/100 g	Whole milk	Skim milk	Sheep milk	Human milk
Thiamine (B_1)(mg)	0.045	0.038		0.15
Riboflavin(B_2)(mg)	0.175	0.17	0.62	0.38
Niacin (B_3)(mg)	0.09			
Pyridoxine (B_6)(mg)	0.05	0.05		0.14
Pathothenic acid (mg)	0.35	0.28	0.35	0.21
Biotin (μg)	3.5	5.0	9.0	0.58
Folic acid (μg)	5.5	300		8.0
Vitamin B_{12} (μg)	0.45	0.4		0.05
Vitamin C (mg)	2		4.3	6.5
Vitamin A (μg)	40	2.4	0.43	71
Vitamin D (μg)	0.03	trace		0.073
Vitamin E (μg)	100	trace		278
Vitamin K (μg)	5	0.01		0.296

Adapted from Kailasapathy (2008) and Souci (2008)

Trace elements from various sources are found in milk, possibly from the feed, contamination after milking, and during milk processing. Zinc the highest concentration in milk (3 mg/kg). Other elements include silicone, fluoride, manganese, and iodine. Manganese is an important element for lactic acid bacteria metabolism, particularly citrate fermentation. Other minor components in milk include organic acids in the milk serum, nitrogenous compounds, gases (O_2, CO_2), hormones such as prolactin, and steroids.

Milk Enzymes

There are several enzymes in bovine milk. They occur in various states including free in solution, as associated or integral parts of membrane fractions of the fat globule membrane or membrane vesicles (in skim milk), and associated with casein micelles or microsomal particles. Approximately 60 indigenous enzymes are excreted from secretory cells in the mammary gland including lipoprotein lipase, plasmin, phosphatases, lactate dehydrogenase, catalase, lactoperoxidase, and xanthine oxidase. Several have important functions in dairy processing and quality control including lipoprotein lipase, plasmin, and alkaline phosphatase.

Esterases also occur in milk. Three types have been determined: the A-type carboxylic ester that hydrolyzes aromatic residues, the B-type that hydrolyzes alphaphilic esters rapidly and aromatic esters slowly, and the C-type that hydrolyzes alkaline esters.

Several enzymes in milk have antibacterial functions including lysozyme, lactoferrin, and lactoperoxidase. The enzymes with major functions in milk and milk processing are discussed below.

Lysozyme

Lysozyme consists of 148 amino acids and it is a relatively small, single polypeptide protein. It is found in low concentrations in bovine milk (0 to 2 mg/L). This enzyme's level rises significantly during infection with mastitis.

Lactoperoxidase

The lactoperoxidase enzyme consists of 712 amino acids and has antimicrobial activity. Its optimum activity is at pH 6.0, but it is stable over pH 5.0 to 10.0. Higher concentrations of this enzyme are present in bovine milk compared to human milk. It chelates to metals including iron.

Oxidoreductases

Milk contains oxidoreductases such as xanthine oxidase, sulfhydryl oxidase, and superoxide dismutase. Xanthine oxidase is a

dimeric metallo-flavoprotein that associates with the fat globule membrane and is approximately 30 kDa. It is a nonspecific oxidoreductase that catalyzes the oxidation of a variety of substances, including nitrate to nitrite in cheese, which inhibits the growth of butyric acid bacteria. Higher concentrations are present in bovine milk than human milk.

Superoxidase dismutase is located in the milk serum and catalyzes the dismutation of the superoxide anion to hydrogen peroxide and triplet oxygen. It may inhibit the oxidation of milk constituents including the autoxidation of lipids. Sulfhydryl oxidase is associated with lipoproteins in the fat globule membrane. It catalyzes the oxidation of sulfhydryl groups to disulfides and is inactivated partially by pasteurization.

Plasminogen and Plasmin

Plasminogen is the principal proteolytic enzyme in milk. It remains inactive until converted to plasmin via plasminogen activators. There are three plasmin types.

Plasmin is responsible for the production of γ-casein and proteose peptone by hydrolysis of β-casein. It also hydrolyzes many other proteins. Its optimal activity is at 37°C and pH 8.0; however, approximately 82% of the enzyme's activity is lost after pasteurization and is reduced by UV light. The concentration of plasminogen and plasmin is affected by lactation stage, disease status, diet, age, breed, and hormone use.

Lipoprotein Lipase

Lipoprotein lipase is the principal lipase in milk bound mostly to casein micelles. It is a glycoprotein that is approximately 10 kDa, which hydrolyzes triglycerides and diglycerides into fatty acids. Its optimum activity is at 37°C in the pH range of 8.4 to 9.2. It is inhibited by its products (i.e., fatty acids), UV light, heat, acid, calcium chloride, manganese chloride, and oxidizing agents. It is stimulated by the presence of bovine serum albumin (BSA), sodium chloride, magnesium chloride, and calcium.

The fat globule membrane protects most lipids from enzyme activity. However, damage to the membrane results in lipase activity and the production of short-chain fatty acids that increase rancidity and produce off flavors, which render milk unacceptable for consumption. Lipolysis can occur from vigorous agitation and homogenization.

Phosphatases

Two main phosphatases occur in bovine milk: alkaline phosphatase and acid phosphatase. Both hydrolyze phosphoric esters and have roles in the processing of milk and milk products.

Alkaline phosphatase is an enzyme situated in the fat globule membrane that hydrolyzes phosphoric esters. Its optimum activity is at pH 8.5 at 37°C. It is activated by divalent metal ions and inhibited by metal chelators, orthophosphates, and pasteurization. Its concentration in bovine milk depends on the individuality of the cow and the lactation stage.

Acid phosphatase is an enzyme that hydrolyzes tyrosine phosphorylated proteins and aryl and acyl phosphates; it is approximately 42 kDa. It is predominantly located in the milk serum and its optimum activity is at 37°C and pH 7.9. Its concentration in bovine milk depends on the lactation state and disease status of the cow. Its activity is lower than alkaline phosphatise; however, its heat stability is not affected by pasteurization. Its activity is inactivated by UV light, and inhibited by heavy metals, oxidizing agents, orthophosphophosphates, and polyphosphates. It may influence heat stability in dairy products.

Factors Affecting Composition, Quality, and Safety of Milk

Several factors affect the composition, quality, and safety of milk. Environmental, physi-

Table 2.5. Factors that may affect milk quality.

Production	Processing	Distribution	Consumption
Animals	Equipment design	Temperature	Consumer perception
Milking technique	Handling	Handling	Nutrition
Pipelines	Storage	Storage	Handling
Cooling	Cleaning/sanitation	Spoilage	Storage
Farm storage	Packaging	Analysis of returns	Flavor
Equipment design	Quality control	Flavor	
Cleaning/sanitation	Transportation		
Quality control	Flavor		
Microbiology	Odor		

Adapted from Kailasapathy (2008)

ological, and genetic factors influence the composition of milk as do extraneous factors such as pesticides, antibiotics and dust. Various environmental factors also affect the quality and safety of milk (Table 2.5).

The composition of both human and bovine milk is influenced by several factors including the lactation stage, diet, location, age, disease status, and individuality. Specifically, the composition of bovine milk is affected by several other factors including the breed, species, pregnancy, season, parity, and processing. Several factors are discussed more thoroughly below.

Species

Milk originating from various milk animals including buffalo, goat, cow, and sheep is used for human consumption. The composition of the milk varies between species, particularly in relation to the concentration of fat and the types of fatty acid residues present. For example, horse's milk has higher protein concentration than human milk, but lower protein concentration than cow's milk. The fat content in horse's milk is lower in concentration than human and cow's milk (Malacarne et al., 2002). Furthermore, the concentrations of conjugated linoleic acids varied significantly between goat milk (average 0.48 g/100 g) and sheep milk (average 0.82 g/100 g) (Talpur et al., 2009).

Breed

Milk originating from different breeds has shown differences in fat content. Overall, milk from Guernsey and Jersey cows has higher fat content than that from the Holstein cow breed. Similarly, the White Thari cow breed produced higher amounts of saturated fatty acids than the Red Sindhi cow breed and lower concentrations of mono-unsaturated fatty acids, polyunsaturated fatty acids, and conjugated linoleic acids (Talpur et al., 2006). The conjugated linoleic acids and unsaturated fatty acids of goat (Kamori and Pateri) and sheep (Kachi and Kooka) breeds were shown to be significantly different (Talpur et al., 2009).

Individuality

The individuality of animals in a particular breed affects the composition of milk. It has been shown that individual cows in a breed show greater variety in milk composition when compared with individual cows between breeds.

Feed/Diet

The composition of the diet and form in which it is delivered to cows has been shown to have an effect on composition and milk yield. High fat and/or low roughage diets have been shown to reduce the fat content of

milk. Overall, the influence of diet on protein and lactose content in the milk has been minimal. Seasonal and regional changes have been shown to influence changes in diet, especially severe heat. However, generally slight but well-defined variations are present in both the fat and solids-not-fat components of milk over the course of a year.

Comparisons between concentrate-fed and pasture-fed milk animals, including goats and sheep, showed that different feeds affected the composition. Higher levels of conjugated linoleic acids (CLAs) and polyunsaturated fatty acids (PUFAs) were present in the milk of pasture-fed cows, goats, and sheep (Morand-Fehr et al., 2007).

Stage of Lactation

The lactation stage of the cow influences the milk yield and the concentrations of lactose, fat, and protein in milk. Lactose and fat concentrations increase as lactation progresses.

Milking Frequency

The frequency and completeness of milking affects milk yield and milk composition. Shorter times between milking have resulted in poor milk yield and higher fat content.

Location

The composition of milk is affected by the location of cows. In particular, farming management practices influence the composition of milk. For example, comparisons between conventional and organically farmed cow's milk have shown significant differences in the compositions of fatty acids (Bloskma et al. 2008). The location also influences the feed given to the cows, and this affects the overall composition of milk.

Disease Status

The disease status of the animal contributes greatly to a variable composition, and also affects the safety of milk. Mastitis (severe inflammation of the udder due to pathogenic microorganisms) is the primary disease that contributes to variations in milk composition and milk yield. Consequently, dramatic losses in milk yield and very high counts of lymphocytes occur.

Processing

The processing of milk includes agitation, mixing, and cooling at the farm; clarification, separation, and standardization; pasteurization; homogenization; packaging and distribution; followed by sanitation of the processing area. Clarification involves centrifugation to remove somatic cells, bacteria, and sediment from the milk. Next, cream is separated from skim milk via centrifugation followed by pasteurization (usually 72°C for 15 seconds), which destroys pathogens and other microorganisms. Homogenization is the mechanical process of shearing fat globules via pressure reducing the size of the fat globules and reducing the separation of the cream portion of the product.

The many factors that influence milk composition are eliminated by standardization, which is a processing requirement.

The composition of organic milk may vary from conventional milk because organic processing avoids the use of synthetic fertilizers, pesticides, growth promoters, or additives. Furthermore, organically certified milk does not use any ingredients, additives, or processing aids derived from genetically modified organisms (GMOs). The organic industry strictly forbids the use of ammonium, bleach, and hypochlorite products for the cleaning of processing areas and equipment. The products used should be biodegradable, have low toxicity, and not contaminate the environment. Several studies comparing organic and conventional milk have shown that lipid composition of organic and conventional milk are significantly different. The conjugated linoleic acids and omega-3 fatty acids, in particular, were sig-

nificantly higher in organic milk compared to conventional milk, which has been shown to prevent asthma, cardiovascular disease, and allergies (Bloskma et al., 2008; Lavrencic et al., 2007; Bergamo et al., 2003; Anon., 2008).

Physical Properties of Milk

The physical properties of milk greatly influence the quality of milk, milk products, and the operations during processing, including the fluid flow, heat transfer processes, and emulsification. A summary of the physical properties of bovine milk are shown in Table 2.6.

Physical Structure

The structure of milk can be segregated into three different phases: a dilute emulsion, colloidal dispersion, and a solution. All of the particles in milk exhibit Brownian motion because they have a negative electrostatic charge. The dilute emulsion phase consists of fat globules; the colloidal dispersion phase consists of the casein micelles and colloidal calcium phosphate (salts); and the solution consists of the whey proteins, water, lactose, and dissolved minerals.

Table 2.6. Physical Properties of Bovine Milk

Property	Value
Density (g/cm^3 at 20°C)	1.032
Freezing point (°H)	–0.540 (–0.521°C)
Boiling point (°C)	100.17
Electrical conductivity (mho/cm at 25°C)	0.004–0.005
Specific heat (kJ/kg/K at 15°C)	3.92
Viscosity (cP)	1.9
Surface tension (dynes/cm)	50–52
pH (25°C)	6.5–6.7
Thermal conductivity (J/msK at 37°C)	193
Refractivity index (20°C)	1.3440–1.3485
Osmotic pressure	700 kPa
Specific gravity	1.032
Titratable acidity (mmol/L)	13–20

Adapted from Francis (2000), Kailasapathy (2008), Neville (1995), Sherbon (1988), Walstra et al. (1999)

A physical equilibrium in milk exists between the colloidal dispersion phase and the salts. However, it is influenced by several factors including the concentration of serum solids, addition of ionizable salts or alcohol, heat, and pH changes that all influence the technological behavior of milk during processing.

Electrical Conductivity

Electrical conductivity is a measure of the electrical resistance of solution in reciprocal ohms (mho/cm) that is used to assess the total ionic content of milk. The electrical conductivity of bovine milk at 25°C ranges from 0.004 to 0.005 mho/cm.

Electrical conductivity measurements are used to screen for the presence of diseases in milk, particularly mastitis, and to detect residual cleaning agents including sanitizers. The ions that contribute the greatest to electrical conductivity are sodium (Na), potassium (K), and chloride (Cl). Sodium and chloride ions increase when mastitis is present, leading to an increase in electrical conductivity.

Skim milk has lower conductivity compared to whole milk because it is increased by the presence of fat. Furthermore, the fermentation of milk increases conductivity due to conversion of calcium and magnesium to ionic forms. Conversely, it decreases during the demineralization of whey proteins due to the loss of ionic minerals.

Oxidation-Reduction Potential

The oxidation-reduction potential (Eh), also known as the redox potential, is a measure of the capacity of a chemical species to acquire electrons, which is expressed in volts (V). If a chemical species is oxidized it loses electrons and if it is reduced it gains electrons. The oxidation-reduction potential of milk at 30°C is between +0.2V and +0.3V, which is determined mainly by the amount of dissolved oxygen.

The oxidation-reduction potential can be altered by various factors including the concentration of dissolved oxygen, ascorbic acid, riboflavin, pH, temperature, and cystine-cysteine contents present in the milk. The oxidation-reduction potential of milk can be used to estimate the amount of lactose available for fermentative bacteria because the amount of lactose available correlates with the amount of dissolved oxygen. The oxidation-reduction potential can be determined using the methylene blue reduction test.

Rheological Properties

Rheology is the study of the transition of materials subjected to applied forces, in which a distinction is usually made between fluids and solids. Fluids flow under the influence of forces, whereas the solids stretch, buckle, or break. The rheological properties of milk include viscosity, surface tension, and foaming, which are used in the assessment and monitoring of the quality of milk-derived products including cheese, yogurt, butter, and cream.

Viscosity

Viscosity is the resistance of flow in centipoise (cP) units. Several factors influence viscosity including the temperature, concentration, and state of the casein micelles and fat globules. Casein micelles affect viscosity the greatest. Protein hydration also has resulted in an increase in viscosity.

Viscosity can be used to determine the amount of casein micelle aggregation in milk as well as the rate of creaming, mass and heat transfer, and flow conditions in dairy processes, and in the designing of dairy processing equipment. Viscosity contributes to organoleptic properties including mouth feel and flavor. The viscosity of whole milk is approximately 1.9 cP; skim milk, 1.5 cP; and whey, 1.2 cP at 20°C.

Surface Properties

The surface tension of milk is determined by the work required to increase the surface area of it, expressed as dynes/cm or mN/m. The surface tension of whole milk is approximately 50 to 52 dynes/cm; skim milk, 55 to 60 dynes/cm; and cream, 46 to 47 dynes/cm at 20°C.

The surface properties affect the absorption, formation, and stability of emulsions, which influence creaming, fat globule membrane function, foaming, and emulsifier use in dairy products. Surface tension is influenced by the concentration of casein and temperature. Homogenization, sterilization, and other processes involving heat have been shown to increase the surface tension of milk. Surface tension can be used to follow the changes in surface-active components during milk processing, the foaming tendency of milk, and the release of fatty acids during lipolysis.

Foaming

Foaming properties affect the handling of milk and its incorporation in dairy ingredients. Foam is air cells contained within a protein film matrix. The formation of stable foam depends on the lowering of surface tension that allows the spreading of surface-active components into thin films only if the films are sufficiently elastic and stable enough to prevent the coalescence of the air cells created.

Stable foam is essential to produce the correct overrun and texture in frozen dairy desserts including frozen and whipped yogurts. Foam control is required during processing to reduce the development of foam in pipelines and during heat treatment because the presence of foam can lead to ineffective pasteurization. The minimum amount of foam is produced at temperatures between 30°C and 35°C; however, it increases below 20°C and above 30°C.

Curd Tension

Curd tension is an important factor in cheese making and milk digestibility. The curd tension of milk is 28 to 54 g. Heating of milk and the addition of salts (i.e., calcium) causes a reduction in curd tension.

Thermal Properties

Thermal properties influence the quality, flow rate, and storage considerations for milk. Thermal properties include heat stability and transfer, expansion of milk (coefficient of thermal expansion), heat capacity, specific heat, and thermal conductivity.

Generally, the rate of heat transfer is maximized in dairy processing operations, which results in a better quality product. Heat stability is mainly determined by the protein composition, pH, and various salt concentrations. Heat expansion affects the flow rate and storage conditions during processing treatments (i.e., pasteurization). Milk expands approximately 0.335cm^3/Kg/°C between 5°C and 40°C.

Heat Capacity

Heat capacity is the capacity of a substance to store heat. It is measured in J/kg/K. The heat capacity of skim milk is 3,906 J/kg/K at 50°C and increases linearly to 4,218 J/kg/K at 140°C. The heat capacity of milk and cream depends on the fat content, because high fat content results in a lower heat capacity.

Specific Heat

Specific heat is the amount of heat required to raise a unit temperature of a substance by a certain temperature interval. The specific heat of whole milk is 3.931 kJ/kg/K at 80°C.

Specific heat is influenced by the fat and water content of a product and is used in the dairy industry to determine the amount of heat or refrigeration required to change the temperature of milk.

Thermal Conductivity

Thermal conductivity is the rate of heat transfer through a material. It determines how fast milk is cooled or heated and it increases with temperature. However, it decreases with an increase in fat or total solids. The thermal conductivity of milk at 37°C is 193 J/msK and 223 J/msK at 80°C. Thermal conductivity is frequently exploited to extend the shelf life of milk.

Optical Properties

Optical properties include appearance, flavor, and refractivity. They mostly influence a consumer's perception of a product.

Appearance

The appearance of milk is perceived as a measure of quality, purity, and richness by consumers. The opaque, white color of milk is due to the scattering of light by the fat globules, casein micelles, and colloidal calcium phosphate. The intensity of the color is proportional to the size and number of these particles, which is increased by homogenization. Therefore, homogenized products appear whiter than unhomogenized products. Furthermore, the yellowish color depends on the concentration of carotene, and the greenish color of whey and milk serum depends on the concentration of riboflavin.

Refractivity

The refraction of light is influenced by the concentrations of solutes in solution. Each solute has its own refractivity index. The refractive index of the solute and solvent determines a solution's refractivity. The refractive index of whole milk is 1.3440 to 1.3485. Water has the highest refractive

index, followed by proteins, lactose, and other minor constituents.

Flavor

Taste and aroma influence the assessment of a quality product to consumers. Milk has a clean, pleasantly sweet flavor; however, it is bland and the presence of off flavors is very noticeable. Off flavors can occur by contamination of microorganisms, processing conditions, and undesirable chemical or biochemical reactions.

Colligative Properties

Colligative properties depend on the number of dissolved particles, particularly lactose, and the dissolved salts including sodium and chloride in solution, rather than their properties. Colligative properties include freezing point, boiling point, and osmotic pressure.

Osmolarity and Osmotic Pressure

Osmolarity is the total number of particles in a given volume of solution expressed as osmol/Kg. The osmotic pressure of milk is approximately 700 kPa (7 bar), which is influenced by the salt and lactose content of milk. It is the same as the osmotic pressure of blood. Osmolarity is proportional to the freezing point of milk and the osmolarity of infant formulas is controlled to resemble the osmolarity of human milk.

Freezing Point and Boiling Point

The freezing point of milk is –0.540°H (–0.521°C), which is lower than the freezing point of water. This is principally due to the presence of dissolved components such as lactose, sodium, potassium, and chloride. The freezing point is the same for products derived from milk including cream, whey, and skim milk. It is used to determine whether bovine milk has been diluted with water using a cryoscope. If so, the freezing point increases. The freezing point of fermented milk, goat, and sheep milk is significantly lower than that of bovine milk.

Similarly, the boiling point of milk is 100.17°C, which is higher than that of pure water due to the dissolved components lactose, sodium, potassium, and chloride.

Density

Density is the mass/unit volume expressed as g/cm^3. The density of milk at 20°C ranges from 1.027 to 1.033 g/cm^3.

The density is influenced by various factors including temperature and total solid concentrations. An increase in fat content or temperature results in a decrease in density; conversely, an increase in solids-not-fat content results in an increase in density. The solids-not-fat concentration, fat concentration, and density can be calculated if two factors are known, as well as the total solids percentage.

Density is useful to convert volumetric to gravimetric measurements and in the production of cream. The density of fat is lower than that of the other constituents in milk, which ultimately causes the fat portion to rise to the surface of the milk when it is left undisturbed. Mechanical separators are used to separate the cream portion from milk.

Specific Gravity

Specific gravity is determined by the ratio of mass of a solution or substance to the mass of a similar volume of water. The specific gravity of whole milk is between 1.030 and 1.035, averaging 1.032. The specific gravity of milk is used to determine the amount of non-fat solids and the addition of water using a lactometer. The addition of water lowers the specific gravity. The addition of sugar or milk solids in yogurt and ice cream dairy

mixes increases specific gravity. Conversely, an increase in fat lowers specific gravity.

Titratable Acidity and pH

Titratable acidity is the amount of alkali or base required to reach a neutral pH. It is used to determine compliance with cleanliness standards and the amount of lactic acid formed in milk by fermentation. The titratable acidity of whole milk is 13 to 20 mmol/L. Titratable acidity decreases by heat treatment due to loss of Ca^{2+} and rises with increases in lipolysis.

The pH of milk is between 6.6 and 6.8 at 20°C. The pH depends on temperature, and it decreases with ester hydrolysis. It can be used as an indicator of bacterial spoilage or activity. The pH of milk also can be exploited to separate the whey and casein proteins because caseins precipitate at pH 4.6.

Functional Properties of Milk

Several constituents of milk have functional properties both in the formation of other foods, and in the body. Functional components derived from milk include calcium, immunoglobulins, enzymes, lipids, lactose, whey and casein proteins, and bioactive peptides.

Calcium

Whole milk contains approximately 1.2 mg/ml of calcium. It is required in various roles of the body including muscle contraction, blood coagulation, enzyme reactions, hormonal secretion, cell signalling stimulation, and blood pressure control. Calcium also is an important factor in maintaining healthy teeth and bones and for protection against various diseases including osteoporosis, cardiovascular disease, infectious diseases, colon cancer, and kidney stones. Technologically, calcium levels influence curd firmness during cheese making.

Lactose

Lactose is the main carbohydrate in milk. It is a disaccharide used in pelleting operations in the pharmaceutical industry, as an anticaking agent, and as an agglomerating agent in foods including confectionery. Lactose contributes largely to the colligative properties of milk. It can be used as the reducing sugar in the Maillard reaction, which is desirable in various foods including coffee, bread crust, and toast. However, it can contribute to off flavors in milk.

Lactose can be problematic for individuals who suffer lactose intolerance because they lack the enzyme β-D-galactosidase in their gastrointestinal tract. Therefore, lactose is not hydrolyzed, which leads to discomfort and bloating; however, this can be alleviated by consumption of yogurt containing probiotic microorganisms that contain the β-galactosidase enzyme. Further, individuals with galactosaemia are unable to metabolize galactose. This genetic disorder can only be treated by eliminating lactose and galactose from the diet.

Lactulose, Lactosucrose, Lactitol, and Galactose-oligosaccharides

The derivates from lactose—lactulose, lactosucrose, lactitol, and galactose-oligosaccharides—have potential prebiotic properties including enhancing mineral uptake, reducing serum lipids, and reducing the risk of intestinal infections and colon cancer.

Lactulose is formed from heating lactose, in which the glucose moiety is epimerized to fructose. It is recognized as a bifidogenic factor. It is present in heated milk (up to 0.2%) but not digestible, and acts as a soluble fiber that has been shown to alleviate constipation and chronic encephalopathy, stimulate the immune response, enhance

calcium absorption in infants, and stimulate the growth of *Bifidobacterium bifidum* in the lower colon area of the human gastrointestinal tract. Similarly, lactosucrose is recognized as a bifidogenic factor due to its ability to enhance the amount of fecal bifidobacteria and possible effectiveness in modifying the fecal flora in patients with inflammatory bowel disease.

Lactitol is a synthetic sugar alcohol produced by the reduction of lactose. It has been shown to significantly reduce the activity of procarcinogenic enzymes and aromatic compounds in the colon, when 20 g/day was administered. It is used as a sweetener.

Whey Proteins

Whey proteins have various technological functions such as emulsification and stabilizing foam. They also have various health benefits including enhancing fat loss and the immune response, and stimulating glutamine synthesis.

Whey proteins exhibit foam stabilizing properties as they form a rigid film at the air-water interface. Also, whey protein concentrates have been produced in concentrated and dried forms. They contain relatively high proportions of essential and non-essential amino acids required by the body, which has led to the use of whey protein concentrates in health food products including snacks, beverages, and bars.

Whey proteins have been shown to enhance fat loss, protein synthesis, and humoral immune response in adults. The branched amino acids in whey protein stimulate glutamine synthesis, which in turn controls antioxidant defenses and immune function. Immunoglobulins G, G1, M, and A protect the young from infections by pathogenic microorganisms. Commercially available products have been introduced containing immunoglobulins that claim to protect individuals against rotavirus and travelers' diarrhea (Korhonen, 2009).

β-lactoglobulin binds to small hydrophobic molecules, including retinol and fatty acids, via a hydrophobic cleft. It also exhibits antiviral, anticarcinogenic, and immunomodulatory properties, and it is used in high-protein-based beverages and soft drinks (Zayas, 1997; Gill et al., 2000; Korhonen, 2009).

α-lactalbumin is a regulatory subunit of lactose synthase, enabling lactose synthesis in the mammary gland; it also it exhibits anticarcinogenic and immunomodulatory properties (Zayes, 1997; Gill et al., 2000). Similarly, bovine serum albumin (BSA) has been found to have antimutagenic and anticarcinogenic properties (Zayas, 1997; Madureira et al., 2007).

Lactoferrin exhibits many beneficial properties, including antimicrobial, anticarcinogenic, antitumor, anti-inflammatory, immunomodulatory, and antiviral properties. It inhibits a range of microorganisms via the removal of iron in milk serum including *Bacillus, Candida, Streptococcus, Staphylococcus*, *and Shingella* species as well as yeast, fungi, and viruses. It also has been shown to be effective in reducing enteric infections in children by inhibiting the growth of some pathogenic strains and enhancing the growth of beneficial bacteria including *Lactobacillus acidophilus.* It is used commercially to prevent viral infections and contamination of raw meat.

Casein Protein

Due to their many functional properties, casein proteins are used in various foods including bakery food, dairy food, and beverages. Their functional properties include emulsification, whipping, and folding; therefore, they are used in small goods, ice cream, and as cheese extenders. Caseins also have extremely good surface-active properties due to their amphiphilic nature.

Casein micelle precipitation is vital for the production of yogurt, cheese, and fermented

milk. The precipitation or coagulation of casein micelles can be achieved in various ways including isoelectric precipitation, rennet coagulation, polyvalent ion precipitation, alcohol precipitation, and heat coagulation.

Caseins contain significant amounts of the essential amino acids required in the body, especially valine, leucine, isoleucine, phenylalanine, tyrosine, and proline. They contain high amounts of proline residues, which result in a lack of secondary and tertiary structures. They also are rich in lysine and can be used as a supplement in lysine-deficient foods such as cereal and other plant-based foods. All three major casein proteins have been shown to have immunomodulatory properties (Gill et al., 2000).

Functional enzymes in milk have antimicrobial properties and are used in processing applications including lactoperoxidase, alkaline phosphatase, lysozyme and plasmin.

Lactoperoxidase

Lactoperoxidase catalyzes the oxidation of thiocynate by hydrogen peroxide to hypothiocynate, which is a potent antimicrobial agent against a wide range of Gram-positive and Gram-negative microorganisms including *Escherichia coli, Lactobacillus plantarum, Pseudomonas flouorescens, Listeria innocua*, and *Staphylococcus aureus*. The lactoperoxidase system forms the basis for applications as an antimicrobial agent in oral health care products and to enhance the shelf life of perishable foods. It also can be used to determine the presence of mastitis.

Alkaline Phosphatase

Alkaline phosphatase is used commercially as an indicator for the effectiveness of the pasteurization process and the contamination of pasteurized milk because its activity is destroyed by the pasteurization process, which kills pathogens. However, it cannot be used to determine efficient ultra-high-temperature (UHT) milk pasteurization because alkaline phosphatase is only partially inactivated.

Lysozyme

Lysozyme is an antimicrobial enzyme that cleaves the glycosidic linkages between N-acetylmuramic acid and N-acetylglycosamine of bacterial cell walls, ultimately leading to cell lysis. Gram-positive bacteria are more susceptible to the activity of this enzyme due to their simpler cell walls.

Plasmin

The activity of plasmin is important in cheese ripening and the stability of casein micelles in products such as UHT milk and milk protein isolates.

Minerals and Salts

Minerals and salts contribute to the chemical equilibrium, nutritional value, and shelf life of milk. They influence several processes including heat stability, alcohol coagulation, age-thickening of sweetened condensed milk, rennin coagulation, and the clumping of fat globules during homogenization.

Growth Factors

Growth factors are potent hormone-like polypeptides that modulate growth and differentiation of a variety of cell types, mammary development, and neonatal development in the gastrointestinal tract. Growth factors in milk include epidermal growth factor, transforming growth factor, insulin, and prolactin.

Vitamins

Milk is a source of both fat-soluble vitamins (A, D, E, and K) and water-soluble vitamins (B and C). The B vitamins in milk include

thiamine, pantothenic acid, and folic acid. Thiamine is a cofactor in carbohydrate metabolism. B_2 has a role in the oxidation of glucose, fatty acids, and amino acids, as well as purine synthesis. Pantothenic acid has a role in fatty acid metabolism and B_6 has a role in protein metabolism. Folic acid is a growth factor and has a role in DNA synthesis. Vitamin B_{12} is a growth factor and has roles in blood formation and nerve tissue functioning. Biotin is used in carbohydrate metabolism as well as lipid, nucleic acid, and protein synthesis.

Vitamin C is found in low levels in milk. It is required for collagen formation, wound healing, and absorption of non-heme iron, and has a role as an antioxidant.

Specific Lipids

Several lipids in milk have potential beneficial properties including conjugated linoleic acids, sphingolipids, butyric acid, omega-3 and omega-6 polyunsaturated fatty acids, branched fatty acids, and ether lipids. Milk also contains the essential linoleic and arachindonic fatty acids, which are not synthesized by the body.

The concentration of conjugated linoleic acids correlates with the concentration of trans fatty acids in milk. However, the concentrations of conjugated linoleic acids are inversely proportional to the concentration of saturated fatty acids.

Several fatty acids have reportedly prevented tumor proliferation including conjugated linoleic acids, branched chain fatty acids, sphingolipids, butyric acids, and ether lipids.

Specifically, conjugated linoleic acids have been reported to prevent colon and breast cancer, increase bone density, reduce chronic inflammation, normalize blood glucose by increasing insulin sensitivity, and exhibit strong antioxidant properties.

Phospholipids have roles in cell-ion interactions and may improve fat absorption in the gastrointestinal tract. Branched chain fatty acids reportedly prevent tumor formation in several animal models and human breast cancer cells (Parodi, 2003; Wongtangtintharn et al., 2004). Sphingolipids may prevent cardiovascular disease and tumor development. Similarly, omega-3 and omega-6 polyunsaturated fatty acids suppress tumor development and lower plasma cholesterol levels that consequently prevent cardiovascular disease (Parodi, 2003).

These beneficial fatty acids could be an important factor in improving the nutritional acceptability of milk fat in the future. Furthermore, the manipulation of the ratios of saturated fatty acids to unsaturated fatty acids could improve the nutritional value of milk fat.

Enhancement via Culturing Milk

Milk can be cultured, providing consumers with additional beneficial properties. Generally, the microorganisms added to fermented milk are probiotic bacteria, which are defined by the Food and Agriculture Organization/World Health Organization (FAO/WHO) as live microorganisms that when administered in adequate amounts confer a health benefit on the host. They are predominantly lactic acid bacteria, which include *Lactobacillus, Bifidobacterium*, and *Pediococcus* species.

Several health benefits are scientifically established including the alleviation of lactose intolerance and the prevention of rotavirus and antibiotic-associated diarrhea. They have also been shown to treat allergies including atopic eczema, stimulate the immune system, prevent inflammatory bowel diseases, and inhibit *Helicobacter pylori* (Vasiljevic and Shah, 2008).

Bioactive Peptides

Bioactive peptides are derived from casein and whey proteins in various ways: by enzymatic hydrolysis with digestive enzymes,

Table 2.7. Bioactivity of major peptide types.

Bioactive peptide group	Protein precursor	Bioactivity	Systems affected
Casomorphins	β- and α-casein	Opioid agonists, ACE-inhibitory, immunomodulatory	Nervous, cardiovascular, immune
α-lactorphin	α-lactalbumin (α-La)	Opioid agonists, ACE-inhibitory	Nervous, cardiovascular
β-lactorphin	β-lactoglobulin (β-Lg)	Opioid agonists, ACE-inhibitory, smooth muscle contraction (ileum)	Nervous, cardiovascular, digestive
Lactoferroxins	Lactoferrin	Opioid antagonists	Nervous
Casoxins	κ-casein	Opioid antagonists, ACE-inhibitory, some smooth muscle contraction	Nervous, cardiovascular, digestive
Casokinins	β- and α-casein	Antihypertensive, immunomodulatory, cytomodulatory	Immune, cardiovascular
Casoplatelins	κ-casein, transferrin	Antithrombiotic	cardiovascular
Immunopeptides	β- and α-casein	Immunomodulatory	Immune
Phosphopeptides	β- and α-casein	Mineral carriers	Digestive
Lactoferricin	Lactoferrin	Antimicrobial, immunomodulatory	Immune, digestive

Adapted from Korhonen et al. (1998) and Meisel (2004)

by fermentation with bacteria, and by proteolysis with enzymes derived from plants or microorganisms. Table 2.7 shows the major bioactive peptides derived from milk proteins.

Bioactive peptides are claimed to provide various benefits to human health and have various bioactivities including antimicrobial, antioxidant, antihypertensive, antagonist and agonist opioid, immunomodulatory, mineral-binding, and antithrombotic properties. Several peptides exhibit multifunctional activities including lactoferricin, which has antitumor, immunomodulatory, anti-inflammatory, antimicrobial, and opioid properties.

Antimicrobial peptides include lactoferrin B f(18–36), which is derived from the chymosin or pepsin digestion of lactoferrin. It has been shown to inhibit several Gram-positive and Gram-negative bacteria (Bellamy et al., 1992; Recio and Visser, 1999). The antimicrobial peptides, caseinglycopeptide f(106–169) and para-κ-casein f(1–105), are derived from κ-casein by hydrolysis using the digestive enzyme chymosin. They have been incorporated into various products including soft drinks, chewing gums, and toothpaste due to their ability to inhibit cariogenic bacteria (Zayas, 1997; Rutherford-Markwick and Moughan, 2005).

Antitumor peptides have been shown to inhibit the proliferation of tumors and are derived from both whey and casein proteins including lactoferricin, β-lactoferrin f(17–38), α-casein f(90–95), and α_s1-casein f(1–3) (Mader et al., 2005; Otani and Suzuki, 2003; Kampa et al., 1997).

Immunomodulatory peptides either suppress or stimulate the immune system and include isradicins derived from α_s1-casein and β-casomorphins derived from β-casein (Maruyama et al., 1987; Kayser and Meisel, 1996). Antioxidant peptides inhibit the formation of free radicals or scavenge free radicals. Antioxidative peptides include α_s1-casein f(144–149), β-casein f(177–183), and β-casein f(168–176) (Suetsana et al., 2000; Kitts and Weiler, 2003).

Antihypertensive peptides are the most extensively studied milk peptides. They have been shown to alleviate hypertension *in vivo and in vitro*, and consequently commercial products including Evolus (Valio, Finland) and Calpis Sour Milk (Calpis, Japan) are marketed as products containing antihypertensive peptides with the ability to reduce blood pressure in individuals with hypertension.

Other bioactive peptides include opioid, antiviral, mineral-binding (particularly calcium), and antithrombotic peptides. Opioid

peptides can be antagonists that are ligands, which bind to receptors without causing a cellular response; however, they inhibit agonists binding to the receptors that enable a cellular response (Teschemacher, 2003). Opioid peptides that exhibit agonistic activity include casomorphins, α-lactophorin, β-lactophorin, and serophin, which demonstrate morphine-like effects, and opioid peptides exhibiting antagonistic activity include lactoferroxins and casoxins that are able to suppress the activity of enkephalins (Brantl et al., 1981; Meisel, 1986; Antila et al., 1991; Tani et al., 1994; Rutherfurd-Markwick and Moughan, 2005).

The commercial use of milk-derived bioactive peptides is emerging due to the increasing evidence that the bioactivities of peptides derived from milk are the most potent. This emergence is occurring in the functional food and nutraceutical industry.

Functional Dairy Ingredients for Food Applications

Forms

Dairy ingredients for the food industry take a number of forms. They can be compositionally complete or partially rearranged (e.g., milk powder, butter), they can include either one component (e.g., anhydrous milk fat, casein) or components rearranged or reassembled (e.g., varied fat percentage in cheeses, recombined milk products), or they can be modified for specific purposes (e.g., alkali solubilized casein, physical homogenization or transformation as in the case of lactose to lactulose).

Milk Fats

Milk fats such as butter are used as ingredients in pastry and other bakery products and have an organoleptic advantage over plant-derived fats. Bulk butter and butter sheets offer functionality and convenience. For example, butter sheets are easily applied in layered bakery products such as croissants and Danish pastries, whereas large butter blocks are difficult to handle and require tedious wire cutting. Butter sheets are convenient, thin, and individually pre-wrapped; however, they must be handled carefully to minimize melting during layering. A major deficiency of bulk butter is its high melting point; however, fractionated milk fat technology allows butter to be fractionated and the fractions reworked to produce functionally superior milk fat.

For example, soft butter oil has a melting point of 21°C to 28°C and is used in butter shortbread manufacture to inhibit winter bloom. Soft butter oil improves mouth feel, viscosity, and emulsion stability in sauces, soups, and cream liqueurs. The hard butter fraction, with a melting point of 42°C, is used as confectionery butter to replace cocoa butter in milk chocolate. Butter shortening is produced by blending the hard milk fat fraction with standard butter oil. It has a melting point of 38°C and improved plasticized texture, and is used in cookies and muffins. A liquid form of butter shortening is made by blending butter shortening with fresh cream. It has a high melting point (38°C) with superior creaming properties. Mechanical treatment and fractional crystallization allow texture modification in milk fat and this is used in the development of dairy spreads and in color control (e.g., white fat in cheese and coffee whiteners).

Proteins

Casein contains strongly hydrophobic regions and random coils, and is heat stable but not acid stable. Whey proteins are globular, helical structures containing hydrophilic and hydrophobic residues and are susceptible to heat denaturation, but they are stable under mild acidic conditions.

Functional milk proteins include casein, caseinate, whey protein concentrate, total

milk protein (TMP), and milk protein hydrolysate (MPH). A number of technologies such as separation (e.g., enzymatic hydrolysis, heat precipitation, isoelectric precipitation, and membrane filtration), co-precipitation (isoelectric precipitation), and mineral modification (e.g., sodium caseinate for emulsification, calcium caseinate to control viscosity) are used to prepare various functional milk proteins. Caseinates (alkali-treated casein) have random coils with a low percentage of helix structure, hence, limited heat gelation and denaturation, but high viscosity in solution. They also have a high electric charge and are more soluble with a strong preference to interphaces (e.g., fat-water, air-water).

Caseinates can form strong flexible membranes used in edible food coating, packaging, and films. They emulsify free fat in meat emulsions, hence, they allow the salt-soluble part of myofibrillar protein of meat for water binding. High viscosity sodium caseinates are used to create a range of textures (cutable to spreadable) with minimal fat separation (e.g., pates). Calcium caseinate is used to whiten chicken nuggets made from mechanically deboned meat and to improve gel strength in surimi.

Whey proteins are low in proline with many sulphide bonds and are globular with strongly folded structures, and they are sensitive to heat. The globular nature of whey protein enables low viscosity in meat pumping brines. Heat unfolds the globular structure of whey proteins, which enables gelling that is used in meat anolog products. Whey proteins can be fractionated according to solubility, size, and charge into two main fractions, namely α- and β-fractions. The α-fraction mainly contains α-lactalbumin, whereas the β-fraction contains more than 80% β-lactoglobulin and less than 5% α-lactalbumin. Unique functional properties of the β-fraction include solubility in acid solution (pH 4.0), no coagulation in low pH or heating, low lipid content, and high foaming capacity, and it forms uniform gels with water-binding and gel strength.

Total milk protein (TMP) is a co-precipitate and includes casein and globular whey proteins. It is used in reformed poultry products (low-grade poultry meat such as feet, head, and dark thigh meat) to improve color and texture. TMP improves juiciness (in batter, premade emulsion); overcomes frying loss, shrinkage, and dry mouth feel in reformed poultry products; and enables the incorporation of high levels of skin in reformed poultry products without "fatting out."

Controlled enzyme hydrolysis modifies the functional performance (flavor, viscosity, machineability) of milk protein. Milk protein hydrolysates (MPH) increase whipping ability and produce stable foams (marshmallow, mousse, nougat). MPH are used to produce stable aerated foods in the presence of high levels of fat, carbohydrate, and protein (e.g., rich, light and fluffy chocolate and fruit mousses).

Lactose

Lactose is 25% sweeter than sucrose and it does not dominate natural flavors. It is used in the baking industry for crust color (browning) and flavor (caramelization). In formulated powdered products, lactose crystals are slow to take up moisture, hence they reduce caking or lumping. In brewing and baking, lactose is not fermented by conventional strains of yeast; therefore, its contribution to color and sweetness is not destroyed. Lactose-hydrolyzed syrups from permeates and whey are used in confectionery and ice cream.

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Chapter 3

Microbiological Aspects of Dairy Ingredients

Michael Rowe and John Donaghy

Milk is a very nutritious medium not only for humans but, if the extrinsic conditions are favorable, for microorganisms. The position of the udder below and proximal to the cow's rectum means that milk is prone to all of the spoilage and, more importantly, pathogenic microflora excreted from the animal's gut. Man has, of necessity, sought means of preserving the nutritious elements of milk, resulting in a plethora of dairy products, certainly beyond the scope of this chapter. Although the need to employ such preservation methods has diminished in many parts of the world, the range of dairy products has, in many respects, been retained because of the desirable organoleptic properties these impart either on their own or as ingredients in other products. It is axiomatic that if used as an ingredient, although the dairy component contributes its own peculiar microflora, the intrinsic and extrinsic characteristics of the final product largely determine the product's spoilage potential and food safety risk. This chapter confines itself to microbial spoilage and food safety concerns of the main milk and dairy products used as ingredients. It does not attempt to describe the microbiological aspects of their production which are aptly addressed in other chapters of this book.

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Raw Milk

Raw milk can potentially contain a multitudinous range of microflora including bacteria, yeasts, molds, prions, and viruses. This variation encompasses not only the microbial types but also their population sizes. Random amplified polymorphic DNA (RAPD) and subsequent cluster analysis of raw milk microflora indicates that the genetic variability among isolates belonging to the same species is commensurate with the genetic variability among different species. Such molecular approaches, based on direct analysis of DNA or RNA and which do not rely on the prior growth of the microflora, have enabled the dynamics of the raw milk microflora and their temporal changes to be elucidated more precisely. However, a list of the possible microflora mainly based on phenotypic tests is given in Bramley and McKinnon (1990) and Oliver et al. (2005), and a fuller description of the individual species is given in Gilmour and Rowe (1990).

The actual microflora in any consignment of raw milk depends chiefly on the following factors:

- Cow health and incidence of clinical and sub-clinical mastitis
- Cow diet and feeding practices
- Milking procedures and practices
- Milk contact surfaces of equipment
- Sanitation practices
- Husbandry practices (e.g., winter housing and summer grazing of cattle)

Microbial contamination of raw milk mainly arises from the following sources: udder interior and exterior, including the teats, milk contact surfaces, and water used for cleaning and rinsing the milking equipment. Contamination from other sources such as the milker themself and aerial contamination are considered insignificant in comparison and are not dealt with here.

Udder Interior

Milk drawn aseptically from the quarters of healthy animals is generally considered almost sterile. However, animals suffering from clinical or sub-clinical mastitis may excrete pathogens such as coagulase-negative staphylococci, *Staphylococcus aureus,* environmental streptococci such as *Streptococcus agalactiae*, coliform bacteria, and *Mycoplasma* spp. Other pathogens not associated with mastitis may also be excreted such as *Mycobacterium bovis, Brucella abortus, Coxiella burnetii*, and *Salmonella* spp.

Udder Exterior

Much lower contamination of teat and udder surfaces is apparent during the summer months in temperate climates when the cows are out at pasture, compared to the winter, when they are housed indoors. This is due to the heavy contamination of bedding material which can be as high as 10^8 to 10^{10} cfu/g. The microflora of the teat and udder exterior is comprised mainly of *Micrococcus* spp. and coagulase-negative staphylococci but also includes members of the genera *Enterococcus, Bacillus, Clostridium, Yersinia, Aerobacter*, and *Listeria.* Among the *Bacillus* spp. found, *B. licheniformis* and *B. subtilis* are the most common species.

Milk Contact Surfaces and Rinse Water

Bacteria may grow in rinse water still remaining after cleaning, particularly if milk residues are present. Potable treated water, if taken directly from the mains supply, is microbiologically assured. However, if untreated water is abstracted from boreholes, wells, lakes, springs, or rivers it can become contaminated by microorganisms of fecal origin (e.g., coliforms), saprophytic microorganisms from the soil and vegetation (e.g., *Pseudomonas* spp., *Bacillus* spores, and coryneform), and lactic acid bacteria.

Raw Milk Safety

In the United States, one-third of all dairy-related illnesses still involve raw milk (Ryser, 2001). The dangers of drinking raw milk are considered to be of such a magnitude that physicians, veterinarians, and dairy farmers who promote, or even condone such a practice, may be at risk of legal action (Weisbecker, 2007). A study involving sampling bulk raw milk from eastern South Dakota and western Minnesota from 131 dairy herds detected *Campylobacter jejuni*, pathogenic *Escherichia coli*, *L. monocytogenes, Salmonella* spp., and *Yersinia enterocolitica* in 0.2%, 3.8%, 4.6%, 6.1%, and 6.1% of raw milk samples, respectively (Jayarao and Henning, 2001).

Raw milk can potentially be contaminated with a wide range of pathogenic microorganisms, not only originating from the animal itself but also from fecal material which inevitably gains access and from environmental sources such as water (Lafarge et al., 2004). The composition, development, or survival of this pathogenic microflora can be influenced by factors such as geographical location, ambient temperature, underlying disease status of the herd, and local human population. This inevitably determines how the milk is treated and consumed as well as regulations and interventions imposed by local and national authorities. The microorganisms pathogenic to humans that potentially could be present in raw milk are shown in Table 3.1. This table has been designed as a quick

Table 3.1. Pathogenic microorganisms associated with milk and dairy products.

Microorganism	Disease condition	Comments
Bacteria		
Aeromonas hydrophila	Can cause wound infections	Opportunistic pathogen, psychrotrophic
Bacillus cereus	Gastroenteritis	Aerobic sporeformer, some strains psychrotrophic, can produce an emetic toxin but such strains are rare in milk
Brucella abortis, Br. melitensis	Fever, chills, sweating, malaise	Sometimes produces intermittent waves of elevated temperature, hence called "undulant fever"
Campylobacter jejuni	Gastroenteritis	Usually self-limiting but severe complications can arise rarely (e.g., Guillian-Barré syndrome and reactive arthritis)
Chronobacter (formerly *Enterobacter*) *sakazakii*	Meningitis, necrotizing enterocolitis	Usually associated with infant formula, Not heat resistant but grows rapidly in rehydrated feeds
Clostridium perfringens	Gastroenteritis	Anaerobic sporeformer, condition is usually mild because unbound bacteria and enterotoxins are flushed from the intestine, owing to profuse diarrhea
Coxiella burnetii	Fever and severe headache	Disease termed Q-fever, one of the organisms used to define lethality of pasteurization
Escherichia coli	Gastroenteritis, some serotypes (e.g., 0157:H7) can produce severe renal failure	Not all strains pathogenic, genetically promiscuous and potentially can harbor a range of virulence factors
Hafnia alvei	Mild gastroenteritis	Can be present in raw milk and is known to grow on the surface of Camembert cheese
Klebsiella pneumoniae	Gastroenteritis and pneumonia	Contamination is mainly from bedding and water
Leptospira interrogens	Disease varies from influenza type to severe icteric form	Contamination is likely via urine of infected animals
Listeria monocytogenes	Meningitis/septicemia	Psychrotroph, among susceptible individuals (e.g., neonates, pregnant women) mortality rate is high
Mycobacterium avium subsp. *paratuberculosis*	Implicated in Crohn's disease	Very slow growing *in vitro.* may survive pasteurization
Mycobacterium bovis	Tuberculosis	Broad host range, lethality of pasteurization partly based on this organism
Mycobacterium tuberculosis	Tuberculosis	Species adapted as a human rather than animal pathogen, usually spread via aerosols
Pseudomonas aeruginosa	Gastroenteritis	In addition, causal agent of cystic fibrosis
Salmonella spp.	Gastroenteritis, which can be severe	Disease requires ingestion of viable organism and not via a preformed toxin
Staphylococcus aureus	Gastroenteritis	Symptoms produced by heat-stable toxin that can be pre-formed in the milk, poor competitor and growth favored by low a_w
Streptococcus pyogenes	Fever	Disease called scarlet fever
Yersinia enterocolitica	Gastroenteritis	Psychrotroph
Streptococcus zooepidermicus	Septicemia	Condition may be complicated by glomerulonephritis
Molds		
Aspergillus spp., *Penicillium* spp., and *Fusarium* spp.	Cancer	Symptoms arise from consumption/exposure to mycotoxins which are secondary metabolites; animals may consume moldy grain/silage and excrete toxins in their milk
Viruses		
Rotaviruses	Gastroenteritis	Detection in milk prevented by intrinsic antiviral antibodies which are destroyed by acid in human stomach
Hepatitis A	Fever and nausea	Virus eventually affects the liver
Polio virus	Fever and nausea	May cause paralysis
Protozoa		
Cyyptosporidium parvum and *minis*	Diarrhea	Infection is by ingestion of oocysts
Entamoeba histolytica	Intermittent diarrhea and constipation	Infection is by ingestion of cysts
Giardia intestinalis	Gastroenteritis	Infection is by ingestion of cysts
Toxoplasma gondii	Spontaneous abortion or congenital defects	Mostly causes asymptomatic infection
Algae		
Prototheca spp.	Wound infection	Especially affects immunocompromised individuals

reference and hence the microorganisms are largely organized in alphabetical order. A more detailed discussion of the major pathogens follows.

Coliform organisms such as *Escherichia coli* are used in many foods and water as indicators of fecal contamination and hence hygiene, but it is well recognized that they cannot be used for this purpose for raw milk. Coliforms can rapidly grow in moist, milky residues in milking equipment, which can then represent major foci of contamination (Singh and Bennett, 2002).

The following section is a more detailed description of selected pathogenic microorganisms potentially found in raw milk. Pathogens found in raw milk but which have particular relevance to other dairy products have been excluded and dealt with elsewhere in this chapter.

***Brucella abortus* and *Br. melitensis*.** *Brucella abortus* primarily affects cattle and *Br. melitensis* affects goats and sheep, though both are pathogenic to humans. The disease presents in various forms and should be included in the differential diagnosis of arthritis, particularly in children. Human infections arise through contact with infected animals or their discharges, including milk and milk products.

Infected animals can be detected through serum antibody tests (e.g., compliment fixation and ELISA or the blue ring test for milk). A PCR assay based on the *omp25* sequence is also available. A useful reference is PHLS Surveillance Centre (1995). Consumption of unpasteurized milk (Foley et al., 1970), cream (Barrow et al., 1968), and cheese (Young and Suvannoparrat, 1975) has accounted for approximately 10% of all reported brucellosis cases, with the remainder occurring primarily among veterinarians, farmers, and meat processors (Wallach et al., 1997).

Coxiella burnetii. *Coxiella burnetii* is a small obligate intracellular Gram-negative bacterium. Antigenic phase I is the virulent form. The organism exhibits a developmental cycle with two size variants which exhibit different antigens. This may explain the high resistance of *C. burnetii* to chemical agents. Clinical presentation is usually pneumonia and/or hepatitis. It can be isolated from vaginal mucus, milk, feces, urine, and semen of infected animals; however, infection is usually via the aerosol route. It is highly infectious. Interleukin 10 seems to play a key role in chronic infection. The organism can be detected using a PCR assay based on the *IS1111* transposon-like region. Bearing in mind the volume of raw milk consumed worldwide, the reports of milkborne Q fever are much fewer than expected (Ryser, 2001). For a review, see Raoult et al. (2005).

Pathogenic *Escherichia coli.* Shiga/verotoxin producing *E. coli* strains have been associated with disease ranging from diarrhea to hemolytic uremic syndrome (HUS). This is mostly associated in the European continent with O157:H7, but other serotypes can be involved e.g., 26, O111. Contamination of raw milk is mainly via feces, though pathogenic *E. coli* strains can cause mastitis in which case it is directly excreted in the milk. Dairy isolates tend to harbor Shiga toxin 1 with a minority possessing intimin (*eae*), serine protease, and/or catalase virulence factors. Because of the promiscuous nature of the species, a wide and varied range of virulence factors can be harbored including the putative factors, cytotoxic necrotizing factors, and cytolethal distending toxins. Therefore, pathogenicity is best determined by multiplex PCR assays directed at individual virulence factors. The serotype O157:H7 is clearly of concern to the dairy industry since it has been detected in 2% to 5% of the raw milk supply (D'Aoust, 1989; Wells et al., 1991), and more than 60 attributed cases are associated with the consumption of raw milk (Ryser, 2001). For a review of the organism, see Bell and Kyriakides (2002).

Campylobacter jejuni. *Campylobacter jejuni* is considered one of the main causes

of infectious diarrhea in developed countries (Garenaux et al., 2005). Symptoms resembling flu develop in approximately one-third of patients sufficiently ill to seek medical attention. This usually occurs 2 to 3 days before the onset of diarrhea, which is sudden and can be severe (Ryser, 2001). Although most patients spontaneously recover within 3 to 7 days, in a small number of cases severe complications can result, namely Guillian-Barré syndrome and reactive arthritis (Nachamkin and Guerry, 2005).

Raw milk is one known vehicle of transmission, and there have been many cases of campylobacteriosis amongst school children on field trips and farm visits involving consumption of raw milk. Contamination of raw milk is mainly via the fecal route, though *Camp. jejuni* is an infrequent cause of mastitis in which case the organism is excreted directly. Numerous outbreaks of food poisoning have been reported in the United States. For example, between 1978 and 1996 there were 111 outbreaks affecting 9,913 known individuals (Friedman et al., 2000). The organism is sensitive to heat (inactivated by pasteurization), acid (pH less than or equal to 5.0), oxygen, and dehydration. *Campylobacter jejuni* can be detected using a PCR assay based on a 286-bp fragment of the *mapA* gene specific to the species (Inglis and Kalischuk, 2004).

Helicobacter pylori. *Helicobacter pylori* is a microaerophile that causes gastritis and peptic ulcers and is a key factor in the development of gastric cancer, gastric lymphoma, and nonulcerative dyspepsia in humans. Its best known genotypic virulence factors are cytotoxin-associated gene A (*cagA*) and vacuolating cytotoxin gene A (*vacA*). It can be detected using a nested PCR based on the phosphoglucosamine mutase gene (*glmM*) or in humans using the C^{13}-urea breath test. For more information, see Fujimura et al. (2002).

Listeria monocytogenes. *Listeria monocytogenes* is a psychrotroph that is responsible for meningo-encephalitis and an intra-uterine infection that causes high mortality in the affected fetus or newborn child. Listeriosis outbreaks have been linked to consumption of raw milk and raw milk cheese. Cattle may be infected via poorly made silage. It can be detected using a real-time PCR assay using primers to amplify a 149-bp fragment from the metalloprotease gene (*mpl*) (Vanegas et al., 2009). The organism has been detected in raw bulk milk at a frequency of 1% to 12%.

***Mycobacterium bovis*.** *Mycobacterium bovis* is acid fast at some stage in its life cycle; as a result, after initial staining it resists decolorization with dilute mineral acid. It has a broad host range covering domestic and wild animals. Consumption of raw milk or products made from raw milk is thought to be the main route of transmission to humans. Many countries operate control programs for cattle with varying degrees of success.

Human infection can result in a range of clinico-pathological outcomes. In the majority of cases the host inhibits proliferation of the organism and no clinical signs of disease are apparent. However, if the host is challenged, e.g., by some other disease process, the organism will resume growth, ultimately resulting in the death of the host. Culture is the primary method of detection, and it is usually preceded by a decontamination step. PCR assays are available, targeting the insertion element *IS6110* and antigen genes such as *mpb70* and *mpb64*. *Mycobacterium bovis* is still considered an important cause of nonpulmonary tuberculosis, with 109 cases reported between 1977 and 1981 in southeast England (Collins and Grange, 1983) and 1 to 5 cases reported annually in Ireland from 1983 to 1994 (Cotter et al., 1996). For more information, see Rowe and Donaghy (2008).

***Salmonella* spp.** *Salmonella* spp. are Gram-negative bacteria that are not usually psychrotrophic. Cattle shedding the organism are often asymptomatic and difficult to identify. The organism can be shed fecally and

contaminate raw milk, which can still exhibit microbiological quality parameters within an acceptable range. Food poisoning arises from ingestion of the viable organism rather than the preformed toxin and results in vomiting, abdominal pain, and diarrhea, and only rarely becomes septacemic. Culture enrichment in buffered peptone water may be required prior to real-time PCR using primers that target a 262-bp fragment of the *Salmonella*-specific *invA* gene (Andrews and Baumler, 2005). It has been detected in raw bulk tank milk at a frequency of up to 9%. It should be recognized that a high percentage of human salmonellosis cases arise from raw milk or raw milk products (CDC, 2002, 2003).

Staphylococcus aureus. *Staphylococcus aureus* is a Gram-positive coccus that is generally a poor competitor but can predominate under low-water-activity conditions such as brine solutions. Food poisoning is usually through ingestion of pre-formed heat-stable enterotoxins of which a wide range can potentially be produced. Symptoms include vomiting and diarrhea. *Staphylococcus aureus* can cause bovine mastitis, in which case the organism is excreted directly in the milk. Some mastitis strains have been shown to be methicillin resistant (MRSA). PCR assays target the *fmhA, femA,* or catalase genes. Perhaps the largest dairy-related staphylococcal food poisoning outbreak occurred in Japan in 2000 when approximately 13,400 cases were attributed to the consumption of powdered skim milk that had been inadvertently contaminated with raw milk (Ryser, 2001).

Yersinia enterocolitica. *Yersinia enterocolitica* is a small ovoid Gram-negative bacillus that is slightly acid fast. Human symptoms include mesenteric lymphadentis simulating acute or sub-acute appendicitis that affects mainly male school children aged 5 to 15 years. Infection is usually as a result of contact with animals and birds or their products, including milk and milk products. PCR assays target the *ail* (chromosomally encoded) and *virF* (plasmid encoded) genes, which are capable of distinguishing the pathogenic (pYV^+) strains of serotypes 0:3, 0:8, and 0:9 from the less pathogenic (pYV^-) serotypes. In addition, a 163-bp amplicon from the *ail* chromosomically encoded attachment and invasion gene can be used for detection (Lliev and Najdenski, 2008).

The organism is killed by proper pasteurization. Nevertheless, its ability to survive for protracted periods on surfaces such as refrigerated milk cartons (Stanfield et al., 1985) has resulted in a number of outbreaks of yersiniosis caused by pasteurized milk with the organism gaining access as a post-pasteurization contaminant either directly or indirectly from surfaces contaminated with raw milk (Auliso et al., 1982; Greenwood et al., 1990). For more information, see Nesbakken (2006).

Raw Milk Spoilage

In some countries, lactococci and coliform growth is favored if milk is stored in unrefrigerated cans and transported to the dairy plant or collection center on the same day, resulting in souring of the milk. This is especially the case for small producers. In many countries milk is refrigerated to 7°C (or 5°C or less if possible) within two hours of milking, held at the farm, transported to the creamery, and held at a temperature under 7°C at the site prior to pasteurization. Holding the milk under these conditions exerts a selective pressure favoring the growth of the psychrotrophic microflora.

Psychrotrophs differ from psychrophiles; the former have an ambient (greater than 20°C) optimum growth temperature but still are able to grow well at refrigeration temperatures (under 10°C). Although the psychrotrophic microflora initially may constitute a small proportion of the microflora (less than 10%), they can predominate after two to three days at refrigeration temperatures (Shah, 1994). This does not induce spoilage

of the raw product unless the refrigerated storage of raw milk is protracted; however, its impact can be significant for products made from that milk.

The psychrotrophic microflora, in particular *Pseudomonas* spp., can secrete extracellular enzymes (e.g., proteases, lipases, and phospholipases) in their stationary growth phases, which may be heat stable and survive pasteurization. This is even true of ultra-high temperature (UHT) heat processing in the case of proteases, subsequently resulting in spoilage of product. The enzymes may be produced in the stationary growth phase, as opposed to constitutively, and bulk milk arriving at a creamery from a transport vehicle usually represents a composite of a number of producers. As a result, a rapid count, even a rapid count of psychrotrophs if that were possible, would not give a reliable indication of the spoilage potential of that milk.

The majority of the *Pseudomonas* species are *Ps. fluorescens* and *Ps. putida* (Dogan and Boor, 2003). Another group of organisms that may be present in raw milk and have the potential to spoil dairy products are spore-forming bacteria, in particular *Bacillus* spp. and *Paenibacillus* spp., and to a lesser extent *Sporosarcinia* spp. (Huck et al., 2008). The ability to form endospores gives them the ability to survive pasteurization and, if the conditions are favorable, to germinate and grow, causing spoilage.

Various interventions have been proposed to minimize the spoilage potential of raw milk and maximize flexibility in processing, including activation of the intrinsic lactoperoxidase system, thermization, addition of carbon dioxide, and low temperature (approximately 2°C) storage.

Lactoperoxidase. Lactoperoxidase is a natural antimicrobial system designed to prevent colonization of the calf's intestinal tract with pathogenic bacteria. It can be activated by the addition of potassium thiocyanate and hydrogen peroxide. The system has been shown to be a safe method for preserving milk when used according to the Codex guidelines either alone or in combination with other approved procedures. It is particularly suitable when technical, economical, and/or practical reasons do not allow the use of cooling facilities for maintaining milk quality (Asaah et al., 2007). The lactoperoxidase system elicits antimicrobial activity against a wide range of microorganisms including bacteria, yeasts, molds, viruses, and protozoa. It does not result in any significant adverse effects on the physicochemical or sensory characteristics of raw milk or processed dairy products and presents no significant toxicological risks. For more information consult FAO/WHO (2006).

Thermization. Thermization is a heat treatment less severe than pasteurization, typically 63°C or 65°C for 15 to 20 seconds. It is designed to kill the psychrotroph population and, with prompt refrigeration, allow extended storage at the creamery prior to processing (Griffiths et al., 1986). Because the time-temperature combination cannot reliably destroy *M. bovis* or *C. burnetii*, it cannot be used as a replacement for pasteurization. Thermization has been shown to result in higher cheese yields and reduction in off-flavor development in both hard and soft cheeses.

Addition of carbon dioxide. The addition of carbon dioxide up to a concentration of 30 mM/l has been advocated to inhibit the psychrotroph population and prolong the safe storage of raw milk. Higher concentrations have been shown to lead to protein instability. Carbon dioxide has been shown to prolong the lag and exponential growth phases of psychrotrophs stored at 10°C and below. It has also been shown to have a differential effect on *Ps. fluorescens*, the psychrotroph most implicated in spoilage, with extracellular enzyme production being inhibited to a greater extent than bacterial growth and final cell numbers. Addition of CO_2 to approximately 30 mM/l was found to reduce

the requirement for rennet during cheese manufacture and result in an increase in grading score compared to untreated controls (McCarney et al., 1995).

Pasteurized Milk

Pasteurization has been defined by the International Dairy Federation as a process applied to a product with the object of minimizing possible health hazards arising from pathogenic microorganisms associated with milk, by a heat treatment which is consistent with minimal chemical, physical, and organoleptic changes to the product. The lethality of pasteurization was originally directed at *M. bovis,* one of the causative agents of human tuberculosis, but was later increased to take account of *C. burnetii*, the causative agent of the human disease Q-fever (Boor and Murphy, 2002). Pasteurization can either be performed using the holder process (i.e., 62.8°C and not more than 65.6°C for at least 30 minutes before immediate refrigeration [less than 10°C]), or more frequently, by the high temperature short time (HTST) process (i.e., not less than 71.7°C for at least 15 seconds before immediate refrigeration). If the fat content of the milk product is 10% or more, if it contains added sweeteners, or if it is concentrated, the temperature is increased by 3°C. Milk also can be subjected to ultra pasteurization, which involves heating the milk to not less than 138°C for 2 seconds and results in a product with a longer shelf-life but still requiring refrigeration. In central Europe pasteurization often involves a heat treatment of 74°C for 30 to 40 seconds, whereas in other European countries, a brief heat treatment at 85°C is used to give an equivalent kill (Muir, 1990).

Bacteria generally exhibit greater heat resistance when in the stationary growth phase compared to the lag or log growth phases, and can also exhibit the phenomenon of cross-protection. This phenomenon can occur when an organism receives a non-lethal primary stress (e.g., acid) which can induce increased resistance to an unrelated secondary stress (e.g., heat) (Rowe and Kirk, 1999). This has implications for the safety margins of pasteurization, which must not only account for variation of heat resistance amongst strains, but also the prior incubation conditions of the organisms.

The effectiveness of pasteurization is usually monitored by measuring the activity of alkaline phosphatase, which is an intrinsic enzyme present in milk and is inactivated by pasteurization (Allen et al., 2004). The residual phosphatase activity of properly pasteurized milk should be less than 100 mU/l.

Pasteurized Milk Safety

Pathogenic bacteria can be present in pasteurized milk through survival of the pasteurization process or as post-pasteurization contaminants. In the case of the thermoduric organisms, two species are of interest: *Bacillus cereus*, which survives pasteurization as endospores, and *Mycobacterium avium* subsp. *paratuberculosis* (Map), which is reputed to survive pasteurization and has been implicated as the cause, or a contributory factor, in the human condition of Crohn's disease.

Bacillus cereus: The organism *Bacillus cereus* can elicit two distinct syndromes: a diarrheal type and an emetic type, both of which are mediated by toxins. The diarrheal type presents as watery diarrhea and abdominal cramps occurring 6 to 15 hours after consumption of contaminated food. The emetic type is characterized by nausea and vomiting one-half to six hours after ingestion of infected food and is usually associated with rice or foods high in starch; therefore, dairy products are minimally implicated unless recombined into other products with the requisite nutritional requirements to induce toxin production. The rate of germination is influenced by growth conditions upon sporulation as well as genetic predetermina-

tion (Johnson, 1984). Dairy products are responsible for very few cases of food poisoning from this organism, because high numbers are required to elicit the symptoms and these would produce overt spoilage that would deter consumption (Goepfert et al., 1972).

***Mycobacterium avium* subsp. *paratuberculosis* (Map)**: The Map organism is the known cause of Johne's disease (paratuberculosis) of cattle, particularly dairy cattle, which presents as untreatable diarrhea, weight loss, and reduction of milk yield. The disease has a severe economic impact and many countries have control programs in place with varying degrees of success. Vaccination of animals is problematic because of a possible cross-reaction with the tuberculin test for bovine tuberculosis.

The organism has been implicated as a cause or contributory factor in Crohn's disease of humans (Behr and Kapur, 2008). This presents as an inflammation of the gastrointestinal tract which results in weight loss and constipation due to occlusions of the colon that may require surgical intervention. The condition is incurable and severely impairs the quality of life of those who suffer from it and their immediate families. Debate rages on two fronts, first on the contribution, if any, that Map makes to Crohn's disease, and second on the ability of Map to survive commercial pasteurization, even when the holding time is increased from 15 to 25 seconds. The evidence for a causal link between Map and Crohn's disease was sufficiently strong for the UK government to adopt the precautionary principle (Rubery, 2001) and advocate strategies to minimize exposure of the public to the organism. However, the evidence suggests that Crohn's disease is likely to be a multifactorial condition with genetic predisposition involving the *Nod2* gene (Bentley et al., 2008).

Regarding heat resistance, the organism was found to be culture positive when naturally infected milk was pasteurized in pilot scale equipment at the extended holding time of 25 seconds (Grant et al., 2002b) and culture positive in a survey of retail pasteurized milk in the UK (Grant et al., 2002a). Other researchers, however, contest that because of the lethality of the pasteurization process, with regard to Map, and the low numbers of the organism likely to be present in bulk milk, that the risk is negligible. In the authors' opinion, the debate, on both fronts, will be protracted.

***Salmonella* spp.** *Salmonella* spp. are not heat resistant, certainly at the high water activity of pasteurized milk. If present in the product it is due to inadequate pasteurization heat treatments or post-pasteurization contamination (Boor and Murphy, 2002). It is not considered psychrotrophic, although some strains grow at refrigeration temperatures, albeit very slowly. Pasteurized milk has been documented as causing salmonellosis outbreaks in the United States and UK due to *S. typhimuruim*. This is an infective process, in that the viable organism must be ingested, rather than a preformed toxin. As a result it has a longer incubation time than toxin-mediated food poisoning with pyrexial illness, diarrhea, and vomiting of several days' duration and in some instances a systemic infection.

Prion proteins. Precursor infective prion proteins (PrP^{c}) have been detected in milk but not in the bovine spongiform encephalopathy (BSE) associated isoform (PrP^{sc}, Franscini et al., 2006). In the UK, the Southwood Working Party concluded that the risk of transmission of new variant Creutzfeldt-Jakob disease (the human form of BSE) via bovine milk was remote on the basis of experience with scrapie, a similar spongiform encephalopathy affecting sheep. However, the group recommended that infected animals and their milk not enter the human food chain.

One of the problems in making this assessment is that in some cases a mouse bioassay was used for detection of the infective prion

and the sensitivity of the assay has been called into question because of the possible low relative transmissibility of the disease from cows to mice as a result of the species barrier effect (Tyshenko, 2007). Certainly prions are known to be very heat stable and would survive pasteurization or even sterilization processes, so emphasis lies in preventing their access to milk rather than applying a process to inactivate them (Asher et al., 1986). The transmission of prions via milk has not been reported from any country with BSE. This lack of evidence suggests that either milk does not readily transmit infective prions or that its infectivity is too low.

Pasteurized Milk Spoilage

Normally shelf-life at 4°C or 7°C is defined as the incubation time necessary for microbial numbers to reach 10^7 to 10^8 cfu/ml, which is the generally accepted threshold for spoilage. The shelf life can range from 7 to 28 days or even longer, depending on the quality of the raw milk, level of post-pasteurization contamination, and temperature control, especially at packaging and storage. Although post-pasteurization contamination after the holding tube can occur as a result of improperly cleaned and disinfected cooling sections, process lines, valves, and tanks, the main point of entry, according to a number of studies, is at the filling machine.

Contamination is mainly by species of the genera *Pseudomonas* and psychrotrophic *Bacillus,* which eventually cause spoilage, mainly due to their ability to multiply at refrigeration temperatures and degrade the protein and fat components in milk. In addition to pseudomonads, in particular *Ps. fluorescens,* other Gram-negative bacteria can be detected after refrigerated storage such as *Flavobacterium* spp., Enterobacteriaceae, *Alcaligenes* spp., *Acinetobacter* spp., and *Aeromonas* spp. *Pseudomonas fragi,* also found in pasteurized milk, produces a characteristic fruity aroma described as resembling strawberries (Gilmour and Rowe, 1990). Species of the genus *Bacillus* and related *Paenibacillus* are Gram-positive spore-forming bacteria that can survive pasteurization and therefore can be present in the pasteurized product as a raw milk contaminant as well as through post-pasteurization contamination. A sub-typing method based on the *rpoB* gene can be used to track the various strains through the milk processing operation (Durak et al., 2006; Huck et al., 2008).

Both genera have psychrotrophic strains and their multiplication during refrigeration leads to spoilage, which manifests as off flavors. The species *B. cereus* can produce a defect known as "bitty" or "broken" cream that appear as flecks of fat floating on the surface of beverages to which the milk has been added and is apparent mostly during the summer months. This is caused by the production of an extracellular phospholipase enzyme that degrades the phospholipid milk fat membrane surrounding fat globules, causing them to lose their colloidal properties and coalesce.

As a result of a move to a more health conscious society, greater quantities of reduced fat milk such as skim and semi-skim are being consumed. The authors have been unable to find any published information indicating that the nature or speed of spoilage of skim or semi-skim pasteurized milk is significantly different from that of whole milk.

Cream

Cream can be defined as "that part of milk rich in fat which has been separated by skimming or otherwise" (Robinson, 2002). Creams are generally identified by their fat content: half and half (10.5% to 18%), single/light cream (18% to 30%), whipping cream (30% to 40%), double cream (more than 48%), and cultivated or sour cream (10% to 40%). Cream to be used as an ingredient in processing contains 36% to 40% fat.

Creams are also identified through the heat treatment to which they are subjected. This has important implications for the safety and spoilage of cream products and their subsequent use as ingredients. Untreated cream indicates cream or milk from which it is derived, which has not been subjected to any heat treatment. Heat-treated creams include pasteurized cream, which is subjected to a temperature not less than 63°C for 30 minutes or longer or above 72°C for 155 minutes or longer; sterilized cream, which is subjected to a temperature of 108°C or higher for 45 minutes or longer in retail containers; and ultra-high-temperature-treated cream, which is subjected to a temperature of 140°C or higher for at least two seconds.

Cream Safety

As a consequence of the heat treatments applied, outbreaks of food poisoning associated with cream or cream-derived products are rare. A few minor outbreaks associated with farm-produced cream have been reported (Sharp, 1987). Risks to public health may be presented by post heat treatment contamination in the packaging and distribution chain. Cream products have a longer shelf life than retail milk and consequently pathogens contaminating the product post pasteurization have the opportunity to proliferate if temperature abuse occurs.

Escherichia coli 0157 : H7 survived seven to 35 days in sour cream when inoculated to contain 10^2 to 10^3 organisms/ml and stored at 4°C (Dineen et al., 1998). *Listeria monocytogenes* attained populations of 10^6 cfu/ml in whipping cream after eight days of storage at 8°C (Rosenow and Marth, 1987), thus emphasising the need to avoid post manufacturing contamination with such pathogens.

Cream Spoilage

Gram-negative organisms, aerobic sporeformers, and yeasts are the major groups of organisms associated with the spoilage of cream. *Pseudomonas* spp. can hydrolyze fat and cause a variety of taints and/or rancidity. Spoilage of cream by yeasts is well described in the early literature, especially if the cream has been sweetened or soured. When sugar has been added, for example in whipping cream, yeasts such as *Torulopsis sphaerica*, *Torula cremoris*, or *Candida pseudotropicalis* can grow and produce a yeasty odor. Although many yeasts do not ferment lactose and consequently fail to proliferate or only grow slowly in cream, a number of species have been isolated from pasteurized samples of cream (Fleet 1990).

Spores of *Bacillus* spp. can survive pasteurization and other heat treatments applied to cream. Ready-to-eat foods (e.g., *sous vides*) have become popular in many countries. Such foods may contain cream and generally do not receive process treatments to ensure inactivation of spores. Among the *Bacillus* spp. recovered from cream are *B. cereus,* which can induce sweet curdling, *B. licheniformis,* and *B. pumilis,* both of which can grow, albeit slowly, at 8°C (Nissen et al., 2001). In addition to spoilage by *Bacillus* spp., the risk from *B. cereus* enterotoxin production and the presence of toxigenic strains of members of the *B. subtilis* group in cream and dairy products cannot be dismissed (Salkinoja–Salonen et al., 1999; Nissen et al., 2001).

Butter

Butter is a stable water-in-oil emulsion derived from cream as its main ingredient. The term butter is reserved for a product with a milk fat content of not less than 80% but less than 90%, a maximum water content of 16%, and a maximum dry nonfat material content of 2%. For a description of butter-making processes, see Robinson (2002) and references therein.

Pasteurization of the cream is the main critical control point in the butter-making process. Post pasteurization contamination is the most likely source of microbial safety and

spoilage issues within the final product, provided plant operation has not been deficient. Cream used for the traditional batch or continuous butter-making processes is generally heated to 74°C to 80°C for 15 seconds and cooled to 5°C to 8ºC, whereas cream for ripened butters is usually pasteurized at a higher temperature (90°C to 95°C for 15 seconds) or flash treated at greater than 100°C before cooling to ripening temperatures (20°C to 27°C). The heat treatments applied should be sufficient to remove vegetative pathogens, lactobacilli, and other microbial contaminants with thermoduric microorganisms removed with the higher temperatures applied to the cream for ripening. Furthermore, many butters are salted (3% to 13%), which also helps with preservation.

Butter Safety

Butter is generally regarded as a low-risk product with regard to food poisoning, although its use as an ingredient could present growth conditions for pathogens (if present) to proliferate and cause a health risk. The increased use of large containers of butter and other yellow fat spreads in restaurants, kitchens, and homes, where containers are frequently removed from refrigerated conditions (temperature abuse), also leads to the possibility of cross contamination.

Robinson (2002) noted that one cluster of listeriosis in the United States was attributed to butter contaminated by *L. monocytogenes*, and there have been a number of recalls of butter contaminated with this organism. An outbreak of listeriosis in Finland in 1998 and 1999 was attributed to butter contaminated with *L. monocytogenes* serotype 3a (Lyytikainen et al., 2000). All 23 cases (6 deaths) were in a tertiary care hospital that had been supplied with the butter in 7-g packages. The outbreak strain was isolated from the butter-producing equipment and the dairy environment, although no error in plant operation was found. It is believed that a single dose of 4.87 $\log_{10}$ cfu could have been sufficient to cause illness among this particularly susceptible group.

Olsen et al. (1988) and Holliday et al. (2003) reported that *L. monocytogenes* can grow between 7 and 14 days at 4°C, 13°C, and 21°C in butter containing 1.2% salt. Subsequent reduction in numbers of *L. monocytogenes* is generally slower at refrigeration temperatures (Holliday et al., 2003) compared to elevated temperatures. Surveys of butter samples in the United States and Italy found no *L. monocytogenes* in samples tested (Kozak et al., 1996; Massa et al., 1990). Salmonella can grow in butter at 25°C and is not eliminated by refrigeration or freezing (Sims et al., 1970). The same authors reported that NaCl content (1% to 4%) as usually employed in the butter industry was not appreciably bactericidal to *S. typhimurium* var. *copenhagen*. Holliday et al. (2003) found that salmonellae grew in sweet cream salted butter at 21°C after inoculation and temperature abuse at 37°C. *Escherichia coli* 0157 : H7 has been shown to survive in butter and butter production facilities (Abbar and Mohamed, 1987) and growth was observed in whipped salted butter (Holliday et al., 2003). These authors reported a number of factors influencing the growth, survival, and inactivation of bacterial pathogens on butter, yellow fat spreads, and margarine, including fat content, pH, the presence of salt, and the presence of other preservatives.

Butter Spoilage

Commercially produced butter is less prone to microbial spoilage than perhaps homemade products. Microbial spoilage is caused predominantly by psychrotrophic bacteria because butter is normally stored under refrigeration conditions. Like cream, Gram-negative rods, including pseudomonads, may contaminate the product post pasteurization and can cause rancidity due to hydrolysis

of butter fat through lipolysis. Putridity and surface taints result from the proteolytic activities of molds and bacteria growing on the butter surface, for example, *Ps. putrefaciens*. Surface growth may become apparent within 7 to 10 days with a concomitant odor resulting from organic acids. Bacteria may also cause other less common spoilage conditions in butter: malty flavor due to *Lc. lactis* var. *maltigenes*, skunk-like odor due to *Ps. mephitica*, and black discoloration due to *Penicillium nigrifaciens* (Jay, 2000). Some members of the genera *Cladosporium, Aspergillus, Penicillium*, and *Mucor* may be responsible for discoloration on the surface of butter, although with improved sanitation and air filtering in many dairy plants, this is not a major issue.

Buttermilk

Buttermilk has two main variants: conventional buttermilk, which is an aqueous phase harvested as a consequence of churning cream during normal butter making, or cultured buttermilk, which is the result of the fermentation of skim or low-fat milk using lactic co-cultures.

According to the International Dairy Federation Standard 163 : 1992, the requirements for buttermilk are acidity of not less than 0.60% (w/w) expressed as lactic acid, fermented by *Lc. lactis* or its subspecies and biovariants and/or *Leuconostoc mesenteroides* and its subspecies. Minimum counts of specific microorganisms should be 10^7 cfu/g at the time of sale. Normally buttermilk is either used directly as a refreshing drink or by the food industry as an ingredient in bread, biscuits, cakes, ice cream, or processed cheese products. The high level of membrane-specific proteins and phospholipids give buttermilk its unique characteristics. Buttermilk can be tolerated by lactase deficient individuals who are unable to consume milk or ice cream.

Conventional Buttermilk

Conventional buttermilk is a by-product of butter making. Depending on the processing conditions, which may involve heating the cream before ripening at 90°C to 95°C for 15 seconds or 105°C–110°C with no holding, sour cream or sweet cream buttermilk may be produced. Sweet cream buttermilk can be further fermented by mesophillic lactic acid bacteria.

Cultured Buttermilk

Cultured buttermilk is made from skim milk that is heated to 95°C for 5 minutes and cooled to 22°C. It is then inoculated with a mixture of *Lactococcus* spp. (*Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*), which are the main acid producers, and *Lc. lactis* subsp. *lactis* biovar *diacetylactis* and *Leu. mesenteriodes,* the latter of which is responsible for aroma and flavor production. The milk may be enriched with sodium citrate (0.1% to 0.15%). This aids production of diacetyl as well as other flavor compounds such as acetoin and ethanol because the level of intrinsic citrate in milk varies, and during the summer is closely correlated with microbiological quality, particularly the number of *Enterobacter* spp. and *Pseudomonas* spp. Some strains of *Leu. mesenteroides* can produce exopolysaccharides, which enhance the functional properties of buttermilk and bacteriocins that are active against *Listeria* spp. After fermentation (14 to 16 hours at 22°C) the product is warmed to 40°C, homogenized, cooled, and packaged.

Food poisoning cases implicating buttermilk are rare and usually occur when it is used as an ingredient to make a product such as mayonnaise, which requires raw egg that may be contaminated with *Salmonella* spp.

Spoilage usually manifests as loss of texture arising from excessive agitation, improper cooling, or use of unsuitable pumps. In some instances disruption of the gel can occur through the production of carbon

dioxide by citric acid fermenting bacteria, giving rise to the phenomenon of curd floating.

Cheese

Cheese making is a complex microbiological and biochemical process in which details are specific to the cheese variety. Apart from acid-coagulated cheeses, cheese making essentially involves the coagulation of liquid milk by added rennet and by acid developed by starter lactic acid bacteria. Milk, usually from bovine, caprine, ovine, or buffalo sources, is prepared through standardization, pasteurization, bactofugation, microfiltration, or other processes prior to acidification and coagulation. The coagulum is cut, stirred, cooked at an elevated temperature, pressed, salted to encourage syneresis (dehydration), and shaped through pressing. While the nature and quality of the finished cheese is determined largely by the manufacturing process, the ripening phase, during which cheeses tend to be stored at low temperature for two weeks to two years, conveys the characteristic texture and flavor of the particular cheese variety.

Cheese may be used directly as an ingredient in the home, food service, and industrial sectors for the preparation of a variety of culinary dishes, formulated foods, or assembled foods (e.g., omelettes, sandwiches, lasagna, pizza, sauces). Natural cheese is also extensively used for the production of processed cheese products (PCPs) and enzyme-modified cheeses (EMCs) and cheese powders. The use of cheese as an ingredient in other foods generally necessitates some form of treatment, for example, comminution, heating/cooking, cooling, freezing, thawing, or secondary processing such as emulsification, pasteurization, and spray-drying. Secondary processing treatments, especially of cheeses previously manufactured from pasteurized or heat-treated milk, should contribute to improved public safety of such cheese-based products. The risk of pathogen growth or cross contamination through mishandling or temperature abuse in catering, retail, or industrial settings cannot be discounted. As indicated herein, the use of raw-milk cheeses as an ingredient in products can increase the risk of exposure to zoonotic agents.

Disease-causing microorganisms are often found in milk used for cheese making (Table 3.1). Raw milk quality is important in producing all cheeses, particularly those made from raw milk. Low bacterial counts and low somatic cell counts are the key indicators of milk quality; higher counts indicate potential pathogen presence (Donnelly, 2004). Pasteurization of the milk (holding pasteurization, 63°C for >30 minutes; high-temperature-short-time, 71.7°C for 15 seconds) effectively destroys most of the microbial contaminants including pathogenic bacteria (Zottola and Smith, 1991). In the absence of pasteurization, U.S. regulations stipulate that cheese must be held at a temperature of not less than 1.7°C (35°F) for a minimum of 60 days. Furthermore, some cheeses are prepared from thermized milk, in which a sub-pasteurization heat treatment is applied to the milk, primarily to destroy psychrotrophic bacteria. Such milk has been designated unpasteurized (De Buyser et al., 2001) rather than raw, which the authors define as milk that has not been heat treated above 40°C.

The temperatures used during the manufacture of cheese to ripen the milk or cook the curd are not sufficiently high to destroy pathogenic microorganisms; therefore, if raw milk is used in cheese manufacture, some pathogenic microorganisms may survive the manufacturing process and subsequently present a public health risk. The UK Institute of Food Science and Technology (IFST, 2000) has highlighted the potential public health hazards posed by pathogenic bacteria, particularly relating to soft and semi-soft varieties. However, some research has noted

the inhibitory effect of raw milk on the survival of foodborne pathogens (Pitt et al., 2000b).

Cheese Safety

Cheese is most vulnerable to the growth of pathogenic bacteria during the early stages of cheese making, when milk is warm. Therefore, the growth and activity of the starter culture (mesophilic, thermophilic, or a mixture of both), with the concomitant production of lactic acid, other organic acids, and anti-microbial agents, creates an environment inhibitory to pathogens. Lactic acid lowers the pH and inhibits most bacteria eventually, including the starter lactic acid bacteria (LAB).

Typical cheese pH values measured at three to seven days after manufacture are 4.9 to 5.5 in most hard-ripened varieties, and 4.4 to 4.8 in fresh lactic and most soft-ripened varieties. Most pathogenic bacteria can grow at pH 5.6 to 7, some grow at pH less than 5.6, and some may survive but few grow at pH values less than 4.6.

For most cheese varieties the pH profile during manufacture and ripening is an important process control. If the starter culture growth is slow, for example, because of bacteriophage contamination or the presence of inhibitors (antibiotics) in the milk, the resultant slow decrease in acidification may enable the growth of undesirable bacteria. Pathogenic bacteria that survive the early acidification phase may grow when acidity is neutralized during cheese ripening, especially in the surface-mold-ripened varieties. This has been observed in the growth of *L. monocytogenes* parallel to the increase in pH (7.5) during the ripening of Camembert (Ryser and Marth, 1987). In contrast, feta cheese, with similar composition in terms of moisture content, a_w, and ripening temperature to Camembert but with a final pH 4.4, inhibits *L. monocytogenes* growth (Donnelly, 2004).

The control of pathogenic and spoilage microflora depends on good farm/animal management and physical processes applied to cheese milk. The growth or persistence of microbial pathogens is influenced by intrinsic and extrinsic parameters (Table 3.2). The safety of the product and ingredient depends on the dynamic interaction between parameters.

In many cheese varieties, cooking (scalding) is required to reduce moisture, but it may have a minor influence on survival of pathogenic/spoilage organisms. Cheeses produced using mesophilic starter cultures have a maximum cooking temperature of 39°C, which ensures an optimum rate of acid development and moisture (whey) release. The low moisture content and pH of such cheeses, for example Cheddar, strongly influences pathogen survival. Considerably fewer foodborne disease outbreaks are associated with semi-hard and hard (low-moisture) cheeses compared to the high-moisture varieties. Thermophilic cheeses, such as many Italian and Swiss varieties, rely on extended high cooking temperatures (50°C to 55°C) for development of typical texture and are rarely

Table 3.2. Extrinsic and intrinsic parameters influencing cheese safety and quality.

Extrinsic	Intrinsic
Time/temperature of storage	Moisture and salt content
Process controls in place	pH and acidity
Product history	Redox potential
Plant hygiene/sanitation	Presence of competing microflora
Personal hygiene	Presence of antimicrobials (natural or artificially added)
Process temperature	Nutrient availability

associated with foodborne disease cases. High temperature cooking is also applied to some fresh cheeses, such as cottage cheese, in which temperatures of 52°C to 60°C for 1.5 to 2 hours is believed sufficient to kill coliforms and psychrotrophic bacteria (Fox et al., 2000).

Cooking temperatures, pH/acidity, and moisture content of cheeses, together with the length of maturation of the finished product and the fact that they are stored at a controlled temperature, constitute a hurdle system of preservation that acts as a series of control steps to inhibit the growth of pathogenic bacteria. Fresh cheeses, for example cream cheese, cottage cheese, quarg, or ricotta, are unripened cheeses, ready for consumption immediately after production. Such cheeses, which make a significant contribution to the ingredients sector, rely on heat treatment of cheese milk to produce a safe product because no ripening occurs.

Little et al. (2008) have identified other contributory factors likely to cause problems with the microbiological quality of cheese made from raw, thermized, or pasteurized milk. Unsatisfactory quality of cheeses was more frequently associated with premises having poor management and control systems. Appropriate hygiene measures to avoid contamination from the production environment and appropriate temperature control of cheeses are critical for minimizing contamination with and growth of pathogens.

The major pathogens of concern in cheeses are those considered for raw and pasteurized milk. While a number of the bacterial pathogens have been responsible for major and sporadic outbreaks of disease, others have not resulted in cheese-related outbreaks but nevertheless have zoonotic potential through evidence of their occurrence and survival during the manufacturing and ripening processes. Outbreaks, prevalence, and challenge studies associated with cheese are described here for the major pathogens recorded in the literature to date.

Listeria monocytogenes. *Listeria monocytogenes* was initially described as a dairy pathogen, but now it is well known that *L. monocytogenes* is an environmental microorganism that associates with the cool, damp environments that are associated with many food processing establishments. Although not as frequent as many other foodborne diseases, *L. monocytogenes* has the second highest fatality rate (21%) and highest hospitalization rate (90%) of all foodborne pathogens (CDCP, 2001).

A considerable number of human listeriosis outbreaks are attributable to cheese sources (Table 3.3). Many of these occurrences have involved considerable morbidity and mortality. Over the period from 1983 to 1987 in Switzerland, Vacherin Mort d'Or cheese was the cause of 122 cases and 33 deaths from listeriosis. Two different epidemic-associated strains of *L. monocytogenes* serotype 4b were isolated from case patients, unconsumed cheese, wooden shelves, and brushes used in cheese-ripening cellars. The high levels of *L. monocytogenes* detected (10^4 to 10^6 cfu/g) on cheese surface samples indicated contamination and growth of the organism on the cheese during ripening.

Between January and August 1985, 152 cases of listeriosis (*L. monocytogenes* serotype 4b) were reported in Los Angeles County, CA. The overall case fatality rate was 33%, of which 65% were prenatal. Mexican-style soft cheese was epidemiologically and bacteriologically associated with the occurrence of the disease. A follow-up investigation of this outbreak from a pasteurized milk cheese revealed gross environmental and equipment contamination as well as inadequate pasteurization. The widespread distribution of *L. monocytogenes* in the environment and its ability to survive on dry and moist surfaces favors post-processing contamination of dairy products from both raw milk and factory sites (McLauchlin, 1997). Most studies have indicated that *L. monocytogenes* is not sufficiently heat resistant to survive pasteurization of

Table 3.3. Documented outbreaks of foodborne illness by bacterial pathogen associated with cheese, 1980–2008.

Country	Year	Pathogen	No. of cases	Product type	Reference	Milk Status[#]
Switzerland	1983–1987	*Listeria monocytogenes*	122	Vacherin Mont d'Or soft cheese	Bula et al. (1995)	UP
USA	1985	*L. monocytogenes*	152	Mexican-style cheese	Linnan et al. (1988)	P
Denmark	1989–1990	*L. monocytogenes*	26	Blue mold/hard cheese	Jensen et al. (1994)	US
France	1995	*L. monocytogenes*	37	Soft cheese (Brie de Meaux)	Rocourt et al. (1997)	R
France	1997	*L. monocytogenes*	14	Soft cheese (Pont-L'Eveque)	Jacquet et al. (1998)	R
France	1999	*L. monocytogenes*	3	Soft cheese	De Valk et al. (2005)	R
Sweden	2001	*L. monocytogenes*	33	Soft cheese	Carrique-Mas et al. (2003)	US
USA	2001	*L. monocytogenes*	12	Mexican-style cheese	MacDonald et al. (2005)	R
Japan	2001	*L. monocytogenes*	86	Washed-type cheese	Makino et al. (2005)	US
Italy	2005	*L. monocytogenes*	3	Gorgonzola blue-veined cheese	Gianfranceschi et al. (2006)	P
Switzerland	2005	*L. monocytogenes*	10	Soft "tomme" cheese	Bille et al. (2006)	US
USA	1981	*Salmonella typhimurium*	321	Mozzarella cheese	Altekruse et al. (1998)	P
Canada	1982	*S. muenster*	Unknown	Cheddar cheese	D'Aoust et al. (1985)	R
Canada	1984	*S. typhimurium*	>1,700	Cheddar cheese	D'Aoust et al. (1985)	P
Switzerland	1985	*S. typhimurium*	215	Vacherin Mont d'Or soft cheese	De Buyser et al. (2001)	R
USA	1986	*S. heidelberg*	339	Uncured Cheddar	Altekruse et al. (1998)	P
Canada	1998	*S. enteritidis*	700	Cheddar cheese	Ryser (2001)	P
USA	1989	*S. javiana/S. oranienburg*	164	Mozzarella cheese	Hedberg et al. (1992)	P
England and Wales	1989	*S. dublin*	42	Soft cheese	Maguire et al. (1992)	UP
France	1990	*S. enterica paratyphi*	277	Goat cheese	De Buyser et al. (2001)	R
France	1993	*S. enterica paratyphi*	273	Goat cheese	Desenclos et al. (1996)	R
Canada	1994	*S. berta*	35	Farm soft "cook" cheese	Ellis et al. (1998)	R
France	1995	*S. dublin*	25	Mont d'Or cheese	De Buyser et al. (2001)	R
France	1997	*S. typhimurium PT12*	113	Morbier (semi-hard) cheese	De Buyser et al. (2001)	R
USA	1997	*S. typhimurium DT104*	110	Mexican fresh cheese	Cody et al. (1999)	R
France	2001	*S. enteritidis PT8*	177	Cantal hard cheese	Brisabois et al. (2001)	R
France and Sweden	2005	*S. stourbridge*	52	Goat cheese	Espie and Valiant (2005)	UP
France	2006	*S. montevideo*	23	French soft cheese	Dominguez et al. (2009)	R
Switzerland	2006	*S. stanley*	82	Soft cheese	Pasture et al. (2008)	UP
USA	2006	*S. newport*	85	Mexican-style soft cheese	Austin et al. (2008)	UP
USA	2007	*S. typhimurium*	29	Mexican Queso Fresco	CDC (2007)	R
USA*	1983	*Escherichia coli* ETEC 027	170	Brie/Camembert	MacDonald et al. (1985)	P
France	1993	*E. coli* (EHEC 0119:B14)	4	Fromage frais from cows' and goats' milk	De Buyser et al. (2001), Deschenes et al. (1996)	UP
France	1994	*E. coli* (STEC 0103)	4	Fromage frais	De Buyser et al. (2001)	UP
Scotland (UK)	1994	*E. coli* (STEC 0157:H7)	22	Farm cheese	De Buyser et al. (2001)	R
England (UK)	1997–1998	*E. coli* (STEC 0157 PT2)	1	Caephilly-type cheese	Reid (2001)	R

(continued)

Table 3.3. Documented outbreaks of foodborne illness by bacterial pathogen associated with cheese, 1980–2008. *(cont.)*

Country	Year	Pathogen	No. of cases	Product type	Reference	Milk Status[#]
USA	1998	*E. coli* (STEC 0157 : H7)	55	Fresh cheese curds	CDC (2000)	R
England (UK)	1999	*E. coli* (STEC 0157 PT 21/28)	3	Cotherstone cheese	CDSC (1999)	US
Canada	2002	*E. coli* (STEC 0157 : H7)	13	Gouda cheese	Honish et al. (2005)	UP
Canada	2003	*E. coli* (STEC 0157 : H7)	10	Cheese (unspecified)	Anon (2003)	US
France	2004	*E. coli* (STEC 0157 : H7)	3	Goat cheese	Espie et al. (2006)	UP
France	2005	*E. coli* (STEC 026 and 080)	16	Camembert type cheese	Espie et al. (2008)	R
Italy	2006	*E. col*i (EAEC)	13	Pecorino (sheep) cheese	Scavia et al. (2008)	UP
USA	1993	*Clostridium botulinum*	8	Commercial process cheese sauce	Townes et al. (1996)	P
Italy	1996	*Cl. botulinum*	8	Mascarpone cheese dessert	Aureli et al. (2000)	UP
Iran	1997	*Cl. botulinum*	27	Traditional cheese preserved in oil	Pourshafie et al. (1998)	US
England	1981–1983	*Brucella melitensis*	2	Goat/sheep cheese	Sharp (1987)	UP
USA	1983	*Br. melitensis*	31	Mexican queso fresco	Altekruse et al. (1998)	R
Greece	1984	*Brucella spp.*	23	Homemade unripened cheese	Sharp (1987)	US
USA	1985	*Br. melitensis*	9	Mexican queso fresco	Altekruse et al. (1998)	R
Malta	1994–1995	*Br. melitensis*	135	Sheep/goat soft cheese	Ryser (2001)	R
Spain	1994–1995	*Br. melitensis*	81	Fresh cottage-type cheese	Ryser (2001)	R
Spain	2002	*Br. melitensis*	11	Goat cheese	Martinez et al. (2003)	US
Italy	2005	*Br. abortus*	5	Pecorino cheese	Farina et al. (2008)	UP
Canada	1980	*Staphylococcus aureus*	62	Cheese curd	De Buyser et al. (2001)	US
USA	1981	*Staph. aureus*	16	Cheese (unspecified)	De Buyser et al. (2001)	P
France	1983	*Staph. aureus*	20	Farm ewe cheese	De Buyser et al. (2001)	R
England	1983	*Staph. aureus*	2	Cheese (unspecified)	De Buyser et al. (2001)	P
Scotland	1984	*Staph. aureus*	27	Ewe cheese	De Buyser et al. (2001)	R
England	1988	*Staph. aureus*	155	Stilton cheese	De Buyser et al. (2001)	UP
Brazil	1994	*Staph. aureus*	7	Minas-type cheese	Araujo et al. (2002)	UP
USA	2007	*Campylobacter jejuni*	68	Cheese (unspecified)	Aghoghovbia et al. (2007)	R
USA	2004	*Mycobacterium bovis*	35	Mexican fresh cheese	Harris et al. (2007)	UP

#: P, pasteurized; UP, unpasteurized; R, raw; US, unspecified
*Implicated in Denmark, Netherlands, and Sweden outbreaks

milk; with respective D_{10} values of 42 s and 2.7 s reported at 62.8°C and 71.7°C (Mackey and Bratchell, 1989).

Undoubtedly, the largest number of cheese-associated listeriosis outbreaks have resulted from the use of raw milk for cheese manufacture. Soft cheese made from raw milk has been determined as the cause of three outbreaks of listeriosis in France in 1995, 1997, and 1999. In 1995, 37 cases were associated with consumption of a Brie-type cheese, with 11 fatalities. No deaths resulted from a similar type of epidemic caused by two soft cheeses, manufactured by the same establishment, in 1997. The incriminated *L. monocytogenes* strains were serotype 2b in both cases. A different *L. monocytogenes* strain type, serotype 1/2a, was implicated in a raw milk soft cheese consumed on-farm in Sweden in 2001. Thirty-three people suffered from febrile gastroenteritis after consumption of the cheese but no deaths resulted.

Among the at-risk groups for human listeriosis are unborn, newborn, neonates, the elderly, and immuno-compromised individuals. The vulnerability of the unborn was illustrated in an outbreak of listeriosis among Hispanic people in North Carolina. Eleven of the 13 patients were pregnant and each had consumed an illicitly produced Mexican-style cheese contaminated with *L. monocytogenes*. The infection resulted in five stillbirths, three premature deliveries, and three infected newborns (MacDonald et al., 2005). The advice for at-risk groups is to avoid soft cheese such as feta, Brie, Camembert, blue-veined, and Mexican-style cheese or products which may contain these cheeses as ingredients (McLauchlin, 1997).

Different prevalence levels of *L. monocytogenes* across cheese varieties and countries have been reported. Studies on the occurrence of *L. monocytogenes* in or on soft cheeses have shown contamination rates of 0.5% (Farber et al., 1987) to 15% (Beckers et al., 1987). In Sweden, *L. monocytogenes* was found in 42% of soft and semi-soft cheeses made from raw milk and in 2% of cheeses made from heat-treated milk (Loncarevic et al., 1995). Similar levels of occurrence (1.1% to 8.2%) have been detected in soft, semi-soft (un)ripened, blue-veined cheeses in other European countries (Lunden et al., 2004; Manfreda et al., 2005). In the United States, more than 35 class 1 recalls of *L. monocytogenes*-contaminated Mexican-style soft cheeses and 28 similar recalls of imported European soft cheeses were instigated between 1980 and 1998 (Ryser, 2001). Lower levels of contamination (1.5% to 4%) have been recorded in hard cheeses (Lunden et al., 2004). A number of studies have reported the absence of *L. monocytogenes* in mozzarella cheeses prepared from both cow and buffalo milk (Banks, 2006). Recorded levels of *L. monocytogenes* in retail raw milk cheeses in the UK (2004), Ireland (2004), Sweden (1994), and Belgium (2000) were 0.9%, 0.2%, 42%, and 47%, respectively. The prevalence of *L. monocytogenes* in retail pasteurized milk cheeses was 0.2% (UK, 2005), 0.11% (Ireland, 2006), 1% (Spain, 1997 to 1999), and 20% (Sweden, 1994) (Little et al., 2008).

Numerous studies have shown that the survival and growth of *L. monocytogenes* depends on conditions during the manufacture, ripening, and storage of cheese, even when the latter is performed at refrigeration temperatures (Pitt et al., 2000b). Given the sensitivity of *L. monocytogenes* to pasteurization regimes, pasteurized milk cheeses prove a minimal health risk, provided post pasteurization/processing contamination is avoided. Poor hygiene, contaminated equipment/personnel, or brine contamination may introduce *L. monocytogenes* into cheeses (Banks, 2006). Brine contamination presents a risk for soft smear ripened and brined cheeses.

Growth of *L. monocytogenes* in cheese is primarily confined to soft and semi-soft varieties such as blue, brick, and Camembert, where populations can increase to at least 10^6 cfu/g as the cheese attains a pH greater than

6.0 during ripening (Ryser, 2001). While growth is not observed in hard cheeses such as cheddar or Colby, survival and persistence has been observed for 70 to 434 days (Cheddar, pH 5.0 to 5.15) (Pearson and Marth, 1990). *Listeria monocytogenes* survived more than 90 days in Trappist cheese (pH 4.7 to 5.42) and feta cheese at pH 4.6. In experiments in which cottage cheese was manufactured from pasteurized milk and inoculated with 10^4 to 10^8 cfu/ml *L. monocytogenes*, the curd cooking temperature reduced levels to less than 10 to 100 cfu/g. No growth of the *L. monocytogenes* occurred due to lactic acid content and associated lowering of the pH (Ryser et al., 1985). During the manufacture of mozzarella cheese, the curds undergo severe heat treatments (80°C to 90°C for 20 minutes) during the stretching (filatura) process. Challenge studies have indicated that stretching curd at 66°C for 5 minutes or 77°C for 1 minute can effectively control *L. monocytogenes* during the production of mozzarella cheese (Kim et al., 1998).

In a study of pathogen survival in Swiss hard and semi-hard cheeses made from raw milk, with respective curd-cooking temperatures of 53°C for 45 minutes and 42°C for 15 minutes, Bachmann and Spahr (1995) detected only *L. monocytogenes* (of all pathogens tested) after 90 days of aging with growth observed on the semi-hard cheese surface. The very hard Italian Grana cheeses (which have a good microbiological safety record), including Parmigiano-Reggiano and Grana Padana, have extended aging periods (more than 12 to 24 months). This elongated maturation coupled with low water activity (a_w), cheese curd cooking temperature-time of 53°C to 56°C for 85 minutes, and mold-holding temperature of 52°C for 10 hours at pH 5.0 ensures that *L. monocytogenes* do not survive the manufacturing process or are rapidly killed during the early stages of ripening (Yousef and Marth, 1990).

***Salmonella* spp**. Of 20 Salmonella outbreaks implicating cheese since 1980 (Table 3.3), the majority have been associated with raw or unpasteurized cheese milk. During the period from 1980 to 1982, an outbreak caused by *S. muenster* in a raw-milk cheddar was traced to a single farm where one cow was shedding the organism (Wood et al., 1984). A second large outbreak occurred in Canada during 1984 and was linked to the consumption of pasteurized cheddar cheese. The contaminated cheese contained *S. typhimurium* phage type 10, subgroup I and II bacteria. Investigators revealed confounding factors which may have contributed to the infection, such as pasteurization process lapses and contamination from personnel (Donnelly, 2004).

After a substantial number of outbreak-associated cases of salmonellosis occurred in Minnesota in 1989, *S. javiana* and *S. oranienburg* were isolated from 2 of 68 blocks (3%) of pasteurized mozzarella cheese. Again, post pasteurization contamination through handling or factory environment were believed the most likely sources (El Gazzer and Marth 1992). Improper pasteurization was the cause of a previous outbreak associated with pasteurized mozzarella (Altekruse et al., 1998).

More recent outbreaks have been associated with raw or unpasteurized milk used in the production of soft cheeses, in which low-level contamination had gone undetected. Cross contamination of milk from poultry or other animals or lack of systematic surveillance for salmonella may have contributed to the outbreaks. Hard (Cantal) and semi-hard (Morbier) cheeses have been involved in two French outbreaks in which the implicated strains were *S. enteritidis* and *S. typhimurium,* respectively. *Salmonella typhimurium* has been the predominant serotype in outbreaks from several countries, with 10 associated deaths (De Buyser et al., 2001). Fifty percent of immuno-compromised cases died in a French outbreak associated with *S. dublin*. Both *S. typhimurium* and *S. dublin* are frequently recovered from raw milk, dairy

cattle, and farm environments (Marth, 1969; Ryser, 2001). Other strains not typically associated with dairy products and global regions have also been isolated from outbreaks, for example, *S. stanley* from Swiss soft cheese (Pasture et al., 2008).

Raw milk used for cheese making can be a source of salmonella. Isolation rates include 4.7% (US, McManus and Lanier, 1987), 8.9% (US, Steele et al., 1997), 0.1% (Ireland, Rea et al., 1992), 2.9% (France, Desmasures et al., 1997), 0% (Belgium, De Reu et al., 2004), 0% (Switzerland, Stephan and Buehler, 2002), and 0% (US; D'Amico and Donnelly, 2008). Despite the number of cheese-related salmonellosis outbreaks, salmonella are rarely recovered from commercially produced cheeses.

Many studies have failed to isolate salmonella from freshly prepared and aged cheddar (Brodsky, 1984a, 1984b), goat cheese (Mor-Mur et al., 1992), raw milk and fresh cheeses (De Reu et al., 2004; Coveney et al., 1994), and raw and pasteurized ripened and semi-hard cheeses (Little et al., 2008). In follow-up investigations to some of the reported salmonellosis outbreaks, the occurrences within the cheese have been sporadic and levels of salmonella were invariably below 10 organisms/100 g, which may suggest a low oral infective dose. In addition, in many of the outbreaks, the source of contamination in the raw milk has been traced to a single or small number of cows within a herd shedding the organisms in their milk. Therefore, co-mingling of milk and the dilution factor may be considerable.

All salmonella are readily inactivated by standard vat and HTST pasteurization. Inadequate pasteurization or post pasteurization contamination of milk can present an issue for the cheese maker prior to cheese making. Salmonella can grow, albeit slowly, at 10°C to 20°C in fluid milk.

The persistence of salmonella during the manufacture and ripening of a number of cheeses has been investigated. Different strains of salmonella have been shown to grow slightly during cheddar cheese manufacture. In addition to the increase due to entrapment during curd formation, salmonella could be detected in cheddar cheeses produced from cheese milk artificially contaminated with 10^5 cfu/ml salmonella after 9 months of ripening at pH 4.5, 10°C. Naturally contaminated cheese recovered from a previous outbreak and ripened at 5°C contained viable salmonella up to 240 days (D'Aoust et al., 1985).

Leuschner and Boughtflower (2002) have demonstrated the survival of three *S. enterica* serovars—*typhimurium*, *enteritidis,* and *dublin*—during the manufacturing stage of a soft cheese which included a cooking step at 45°C for 4 hours. Low inocula (1 to 10 cfu/ml) were used in the study and the strains could be detected in the final product at concentrations between 1 and 50 cfu/g after a four-week storage period.

The manufacture of mozzarella and cottage cheese, both of which have high cooking temperatures, appears to eliminate salmonella. Eckner et al., (1990) reported the complete inactivation of salmonella during the molding and stretching of mozzarella cheese curd. McDonough et al. (1967) reported similar inactivation of salmonella during cottage cheese curd cooking at 52°C for 20 minutes. However, caution must persist with such products and ingredients open to post pasteurization whereby contaminating organisms may survive even though conditions do not favor growth.

Escherichia coli. Enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and verotoxigenic *E. coli* (VTEC) have all been linked to cheese-related outbreaks and sporadic cases (Table 3.3). While strains of the VTEC group have emerged as the predominant public health concern, strains of EIEC and ETEC have been linked to major cheese-related outbreaks in the United States and Europe (MacDonald et al., 1985; Marier et al., 1973). In the large US multi-state and

international outbreak during 1983, ETEC 027:H20 (a shiga-toxin-producing strain) was isolated from patients and the incriminated cheese. A recurrent contamination problem at the French Brie cheese factory was inferred due to the isolation of the organism in batches produced six weeks apart. Studies have demonstrated the increase in ETEC populations during the first four to six hours of cheddar and Colby-like cheese manufacture, with a slow gradual decrease over a 6- to 12-week period of ripening, depending on ripening temperature (Ryser, 2001).

Escherichia coli serotype 0157:H7 is the predominant foodborne pathogen among the VTEC. Although non-dairy products such as ground beef have been associated with the more serious outbreaks, cheeses have been responsible for outbreaks leading to the development of HUS or even death. In 1998, one of the larger outbreaks, involving 55 laboratory confirmed cases of *E. coli* 0157:H7 infection, occurred in Wisconsin. This was linked to consumption of fresh cheese curds that had inadvertently been produced using vats previously used to produce cheese made from unpasteurized (raw) milk. In Alberta, Canada, unpasteurized Gouda cheese was responsible for an outbreak of 13 *E. coli* 0157:H7 infections in 2002. The organism was recovered from two samples (n = 26) of the implicated cheese; one sample was positive for *E. coli* 0157:H7 104 days post production. A number of outbreak cases had consumed small free samples of the cheese at a local market, indicating significant contamination of the product and/or a highly pathogenic organism. The source of the *E. coli* contamination was not traced during the follow-up investigation.

During the 1990s, a number of outbreaks were reported in the UK that were linked to cheese made from unpasteurized milk (Table 3.3). Although hygiene and sanitation practices at the production plants were satisfactory, there were a number of confounding factors which may have contributed to *E. coli* 0157:H7 in the final product implicated in the outbreaks. These included storage of raw milk at elevated temperature, which enabled bacterial growth, no use of starter culture, and a less than adequate maturation time.

Within Europe, non-0157:H7 VTEC infections are considered equally as important as 0157:H7. Germany (Gerber et al., 2002), Italy (Tozzi et al., 2003), and Denmark (Pierard et al., 1999) reported that more than 40% of confirmed cases of VTEC-related HUS were caused by non-0157:H7 VTEC. In 2005, French raw milk Camembert-type cheeses contaminated with *E. coli* 026 and 080 caused 16 HUS cases and a national and international recall of the entire production of cheeses (Espie et al., 2008).

Different cheese varieties have had evidence of VTEC contamination, based on the presence of shiga toxin (stx) or isolated VTEC (Pradel et al., 2000; Fach et al., 2001; Hussein and Sakuma, 2005; Vernozy-Rozand et al., 2005; Rey et al., 2006; Caro and Garcia-Armesto, 2007; Stephen et al., 2008). Irrespective of the detection methods used, published studies report the incidence of VTEC 0157:H7 and other serotypes in both hard and soft cheeses as consistently low. Furthermore, many reports show low recoveries (13% to 40%) of VTEC from PCR-positive samples; thus, the number of field isolates for further investigation can be limited. VTEC serotypes possessing the *eae* gene and toxin subtypes commonly associated with human disease isolates have been detected in raw milk cheeses (Hussein and Sakuma, 2005; Stephen et al., 2008), although Pradel et al. (2008) found few VTEC strains from dairy foods to contain the *eae*, *espP*, and *kat*P genes that are commonly associated with disease-causing strains.

VTEC has been shown to have the potential to survive not only in high-moisture soft or semi-soft cheeses, but also in hard-ripened cheeses. Although viable VTEC decrease with ripening time (Schlesser, 2004; Luukkonen et al., 2005) in hard cheeses, the pathogen could still be detected after 158 days of ripening of a cheddar cheese. A study

by Schlesser et al. (2006) confirmed that the 60-day ripening period was inadequate to eliminate *E. coli* 0157 : H7 during ripening of cheddar cheese. Caro and Garcia-Armesto (2007) reported the isolation of STEC from Castellano cheese, a non-cooked hard or semi hard variety prepared from ewe's milk, after a 12-month ripening period. Using a laboratory-scale smear ripened cheese produced from raw milk, Maher et al. (2001) reported that *E. coli* 0157 : H7 was able to grow during cheese manufacture and survive during the ripening period up to 90 days. Similarly, Leuschner and Boughtflower (2002) and Arocha et al. (1992) showed that *E. coli* 0157 could survive the soft cheese manufacturing process. *Escherichia coli* 0157 : H7 also survived the manufacture and storage of Camembert and feta cheeses at $2 \pm 1°C$ for 65 and 75 days, respectively (Raamsaran et al., 1998). Montet et al. (2009) reported the growth of acid resistant and non-acid-resistant shiga-toxin producing *E. coli* during the early stages of Camembert manufacture and indicated that the 20-day ripening period for such cheeses may not guarantee a safe product if STEC/VTEC is present in the raw milk that is used.

Staphylococcus aureus. Dairy products, including cheese, are known vehicles of staphylococcal poisoning. *Staphylococcus aureus* is commonly found in milk (D'Amico et al., 2008; De Reu et al., 2002) and dairy products made from either raw or pasteurized milk (Coveney et al., 1994), because it is the main etiological agent of bovine mastitis and it is extensively carried by food industry workers (Younis et al., 2003). *Staphylococcus aureus* has been frequently associated with foodborne outbreaks related to cheese made from raw, unspecified, or pasteurized milk in the European Union (EU) (De Buyser et al., 2003), although Ryser (2001) has indicated the number of dairy-associated outbreaks in the United States has decreased considerably since the 1950s and 1960s.

Staphylococcus aureus has been detected at varying levels in retail cheeses. Levels in French cheeses have ranged from 0% to 23%, with higher prevalence among unpasteurized or raw milk cheeses (Little et al., 2008; De Reu et al., 2002). Raw semi-hard and hard cheeses had prevalence levels of 12.5% (De Reu et al., 2002). No *Staph. aureus* was detected in pasteurized retail semi-hard cheeses (Little et al., 2008).

Staphylococcus aureus foodborne intoxication has been associated with the presence of virulent staphylococcal enterotoxins, which are heat-stable proteins produced by approximately 25% of the *Staph. aureus* strains isolated from food. Enterotoxin production is generally associated with greater than 5 log cfu/ml cell populations in milk. Thus, cheeses made from raw milk under EU regulations (D'Amico et al., 2008) must contain fewer than 5 log cfu/ml at the time of manufacture. If values greater than 5 log cfu/ml are detected, the cheese must be tested for enterotoxin presence.

Staphylococci are eliminated by pasteurization. However, enterotoxin produced in milk can persist through cheese milk heat treatments and lead to intoxication in the final product/ingredient. *Staphylococcus aureus* is seldom found in cheeses made from pasteurized milk or is found only in low numbers (Little et al., 2008). Conversely, *Staph. aureus* may increase 1.5 to 3 log units in a wide variety of cheeses including Camembert, feta, and semi-hard and hard cheeses manufactured from raw milk under normal conditions during cheese making. This can be a 1- to 1.5-log unit increase above normal curd entrapment concentration of numbers. Larger increases in cell numbers can result if starter culture activity is compromised. Therefore, these cheeses are a potential health hazard, unless contamination of milk is low and acidification is optimal (Zangerl and Ginzinger, 2001).

Studies have reported the stabilization of *Staph. aureus* numbers during cheese making when salt has been eliminated, apparently due to inhibition of less salt–tolerant background microflora when salt is present (Ryser,

2001). The increased pH (6.5 to 7.0) and ripening storage temperature (10°C to 12°C) associated with Camembert-type cheeses was found to have a cumulative positive effect on staphylococcal growth (Meyrand et al., 1998). The thermal stress (high cooking temperatures), long ripening times, and low water activity associated with the Italian hard cheese varieties such as Grana Padano and Parmigiano-Reggiano present sufficient obstacles for the proliferation of *Staph. aureus* during production (Ercolini et al., 2005). Bachmann and Sphar (1995) assessed the survival of *Staph. aureus* in semi-hard and hard Swiss cheeses and detected a >5-$\log_{10}$ cfu/g decrease over 24 hours and 60 days for the respective cheeses.

Yersinia entercolitica. Worldwide studies indicate that *Y. enterocolitica* is fairly common in raw milk, with reported levels varying between 4% and 81%. However, many isolates from these studies have been classified as avirulent environmental strains. The predominant virulent strains, 0:3, 0:6, 30, 0:8, 0:10k, and 0:13, have been epidemiologically associated with outbreaks in pasteurized milk or milk products. Many of these outbreaks have resulted from post pasteurization cross contamination (Ryser, 2001; Greenwood and Hooper, 1990).

Although no outbreaks associated with cheese have been reported, De Boer et al. (1986) detected four (4.5%) *Y. enterocolitica* positive samples from Brie and Camembert samples tested and one positive sample from 50 blue-veined cheeses. *Yersinia* spp., including *Y. enterocolitica*, have also been detected in raw milk cheeses (Hamama et al., 1992). Other studies have failed to recover *Y. enterocolitica* from Canadian produced cheddar and Italian cheese (Schiemann, 1978) and commercially produced pasteurized Brazilian soft cheese (Aroujo et al., 2002). Bachmann and Sphar (1995) studied the survival of *Y. enterocolitica* in raw milk hard and semi-hard Swiss cheese. In the hard cheese, the organism was not detectable after the curd cooking stage of manufacture, when initial levels of log 5 cfu/ml were used to artificially contaminate the cheese milk. At similar initial concentrations, *Y. enterocolitica* was recoverable in 30-day-old semi-hard cheeses but undetectable later in the ripening cheeses. A 3-log increase in *Y. enterocolitica* populations was observed when pasteurized milk for Colby cheese manufacture was inoculated to contain 10^2 to 10^3 cfu/ml with surviving cells detected after eight weeks of ripening (3°C). Growth and survival on the surface of ripened Brie (4°C to 20°C) and during the manufacture of Turkish feta cheese have been reported (Little and Knochel, 1994; Erkman, 1996).

Their findings reflect the ability of *Y. enterocolitica* to survive and proliferate in milk and milk products at refrigeration temperatures. However, the organism does not survive pasteurization or even lesser heat treatments of milk and milk products. For example, heat treatment of milk at 60°C for 1 to 3 minutes effectively inactivates *Y. enterocolitica* (Lee et al., 1981), and hence does not survive the cooking step in cottage cheese manufacture (Golden and Hou, 1996). However, given its ability to grow at low temperature and the fact that dairy processing plants may harbor the organism, it is important that post pasteurization contamination is minimized to reduce any risks.

Campylobacter jejuni. *Campylobacter jejuni* enteritis outbreaks are frequently associated with consumption of unpasteurized cow's milk. However, fermented dairy products, including cheeses, are rarely associated with campylobacteriosis. One such sporadic instance was associated with the consumption of locally produced unpasteurized fresh cheese in Kansas in 2007 (CDC 2009). Of the 101 people who ate the cheese, 66% became ill, although all cheese samples tested negative for *Campylobacter*. However, the infrequent isolation of the organism from milk or milk products, even in epidemiologically

linked milk outbreaks, is not uncommon. This can be due to:

- A low level of contamination of the implicated product
- The fact that *Campylobacter* spp. initially present in milk do not proliferate and usually die within a few days
- The use of insensitive detection methods

Although *Camp. jejuni* DNA has been detected in cheese samples (Wegmuller, 1993) by PCR and unconfirmed by culture, the cheese challenge studies to date and the fastidious nature of campylobacters support the assertion that cheese is an unlikely vehicle of transmission for Campylobacter enteritis. In the limited number of surveys of cheese products for campylobacters, none have recovered the organism from French raw milk cheese (Federighi et al., 1999), cheddar (Brodsky, 1984a), or Canadian-produced Brie and Camembert (Medeiros et al., 2008). *Campylobacter* spp. are unable to grow at refrigeration temperatures and low pH, and are readily inactivated at cooking temperatures encountered in cheeses such as cottage cheese or Swiss hard and semi-hard cheeses (Bachman and Sphar, 1995).

Brucella spp. Although major outbreaks of brucellosis with cheese identified as the food vehicle of transmission are rare, there have been outbreaks linked to Mexican Queso Fresco cheese and sheep/goat's milk soft cheese (Table 3.3). The latter outbreaks have been caused by *Br. melitensis* and have generally resulted from the consumption of unpasteurized cheese manufactured in a country with endemic brucellosis. For example, cheese-borne brucellosis in England and Wales (1981 to 1983) was associated with sheep and goat cheese imported from Italy and Jordan. Other cases occurring in England and Wales were linked with a major outbreak in Malta (1995), in which unpasteurized sheep and goat's milk was identified as the vehicle of infection. Contaminated raw goat's milk used to manufacture Queso Blanco cheese was responsible for an outbreak among mainly Hispanic patients in the United States during the 1980s. *Brucella* spp. have been isolated from 7.5% of Mexican cheese samples (Acedo et al., 1997) and 5% of retail soft Mexican cheeses (Ongor et al., 2006). In the latter study, all detected strains (by PCR) were identified as *Br. abortus*. This species and *Br. melitensis* have been detected in raw sheep's milk cheeses in Turkey (Sancak et al., 1993) and in 2.2% of French cheeses manufactured in Iran (Akbarmehr, 2003). The consumption of unpasteurized cheese has been identified as a key risk factor for brucellosis in Iran, which is also applicable to raw milk cheeses produced in regions where brucellosis is endemic. Challenge studies performed with Camembert, Tilsit, and cheddar indicate the survival of *Br. abortus* up to 57, 15, and 180 days post manufacture, respectively. However, pasteurization provides adequate margins of safety for *Brucella* spp. in milk.

Clostridium botulinum. *Clostridium botulinum* outbreaks associated with dairy products are rare and sporadic. Since 1980, three cheese-borne outbreaks have been reported, each resulting from toxin A type poisoning. One fatality resulted from each of the outbreaks, the largest of which was in Iran and involved 27 cases (Pourshafie et al., 1998). A traditional Iranian cheese preserved in oil was the incriminating food source. A commercial Mascarpone cheese was the cause of a *Cl. botulinum* outbreak in Italy in 1996. The cheese had been eaten by all of the patients, either alone or as the uncooked ingredient of the dessert tiramisu. *Clostridium botulinum* type A and the associated toxin were detected in tiramisu leftovers and retail samples of the Mascarpone cheese. It is believed that a break in the cold chain at retail likely caused germination of *Cl. botulinum* spores with subsequent production of the toxin (Aureli et al., 2000a).

Anaerobically packaged cheese products, for example, cheese spreads, may present the

highest risk with respect to *Cl. botulinum*. Germination of spores present in such products can be prevented by proper cheese formulation with regard to salt content, moisture content, pH, nisin addition, and water activity. It is believed an onion-containing cheese spread was responsible for an outbreak of *Cl. botulinum* in Argentina (Briozzo et al., 1983), which was due in part to toxin production in a product having higher than normal water activity. More recently, a canned cheese sauce was traced to eight cases of botulinum type A toxin. The cheese sauce formulation supported *Cl. botulinum* growth and toxin production which was exacerbated by temperature abuse (Townes et al., 1996).

Mycobacterium bovis. Historically, the consumption of unpasteurized milk, or products made from it, were the principal vehicles of transmission of *M. bovis* to humans (de la Rua-Domenech, 2006), a situation that changed dramatically with the introduction of pasteurization. Today in developed countries, one is more likely to find tuberculosis due to *M. bovis* in an aged person who acquired the infection some 30 to 40 years ago before the widespread adoption of pasteurization. However, there is an increasing interest in artisan cheeses, some of which are made from unpasteurized milk. A number of recent cases of culture-positive tuberculosis in the United States have been attributed to *M. bovis.* Epidemiologic investigations indicated the consumption of unpasteurized dairy products, including soft fresh cheese originating from Mexico, may have accounted for these cases. A follow-up survey for the presence of *M. bovis* in such products isolated *M. bovis* from a panela-style cheese (n = 204) (Harris et al., 2007; Kinde et al., 2007).

Jalava et al. (2007) suggest that exposure to cattle or unpasteurized milk/dairy products may be the most significant risk factor for human *M. bovis* disease. Because *M. bovis* has been shown to be very resistant to chemical disinfectants, including acids and alkalis, it is likely to survive for protracted periods in cheese. However, this resistance has been shown to vary by cheese (Keogh, 1971). For example, cheddar, Tilsit, and Bulgarian white cheese contaminated with the tuberculous agent were found to be infective after 220, 305, and 120 days, respectively, and Camembert was found to be infective after 3 months (IDF 1980).

Mycobacterium avium* subsp. *paratuberculosis. The putative zoonotic potential of Map has meant the survival of this bacterium during cheese manufacture and ripening has been investigated. Sung and Collins (2000) determined a D value of 59.9 days for the survival of Map in a soft Hispanic-style cheese prepared under laboratory conditions; Spahr and Schafroth (2001) calculated a D value of 45.5 and 27.8 days for model hard (Swiss Emmentaler) and semi-hard (Swiss Tisliter) cheeses, respectively. The D values estimated for the survival of three Map strains during cheddar cheese manufacture were 107, 96, and 90 days (Donaghy et al., 2004). Each of these cheeses have distinct characteristics in terms of water content, starter cultures, pH, and cooking temperatures, yet in each case the inactivation of Map was slow and gradual throughout the ripening period. Map has been detected in retail cheeses in a number of studies: 4.2% of raw milk Swiss cheese samples by PCR (Stephan et al., 2007) and 5% of retail pasteurized cheese curds (Wisconsin and Minnesota) by PCR (Clark et al., 2006; Ikonomopoulos et al., 2005).

Many food safety agencies worldwide have adopted the precautionary principle toward Map, advocating the minimization of entry into the food chain. Cheeses, especially those derived from unpasteurized milk in regions with a high prevalence of Johne's disease, will be scrutinized further should evidence emerge to substantiate the zoonotic nature of Map.

Cheese Spoilage

Microbial spoilage of cheese is caused principally by Gram-negative bacteria, Gram-positive sporeformers, yeasts, and molds.

The concomitant proteolytic and lipolytic enzymes associated with some of these organisms are responsible for many of the undesirable attributes of spoiled cheese.

Bacterial spoilage can be common during cheese manufacture and ripening, although cottage cheese may be susceptible to bacterial deterioration following its manufacture. This can be due to removal of lactic acid during curd washing, low moisture, and salt content. Many of the Gram-negative bacteria involved in cheese spoilage are psychrotrophic and capable of growth under refrigeration. Pseudomonads are the major psychrotrophs associated with cheese spoilage. Excessive lipase and protease activity and sliminess due to growth on the cheese surface can result from high counts in cheese milk, thereby causing cheese off-flavors. Putrescence, off odors, and discoloration caused by proteolytic bacteria and *Pseudomonas* spp. are common among cheeses such as mozzarella. Furthermore, excessive proteolytic activity of starter culture can lead to the development of a bitter taste in cheeses. In blue-veined cheeses, the excessive growth of lactic acid bacteria (LAB) causes high-acid curds to develop, resulting in brittle cracked rinds and loss of flavor in the curds (Scott, 1986).

Large numbers of Enterobacteriaceae can cause gas bubbles (blowing) in the curd of blue-veined and other cheese varieties, a condition termed gassy cheese. Early blowing is also caused by other coliforms including *Citrobacter*, *Escherichia,* and *Aerobacter*. *Aerobacter aerogenes* has been reported to be responsible for blown tins of Domiati cheese and *Klebsiella aerogenes* can cause early blowing and poor cheese quality in white-brined cheeses (Bintsis and Papademus, 2002). Early blowing typically develops up to and including cheese formation and manifests itself through the production of bubbles throughout the cheese matrix. Some cheeses such as feta and other white-brined cheeses may develop a spongy texture. Early blowing can become more evident during salting of hard cheeses such as Grana Padana, when the cheese floats due to pockets of gas (CO_2) formed by bacterial fermentation of lactose trapped within the cheese curd. High coliform loads may arise from contaminated milk, dirty utensils/plant, and poor hygiene.

Sporeforming bacteria also contribute to spoilage of cheese products. Many can survive pasteurization, subsequently germinate, and experience growth of vegetative cells. *Clostridium* spp. may grow in cheeses with low oxidation-reduction potential, producing a late-blowing defect and off-flavors associated with butyric acid and sulfur product formation. The predominant clostridal species associated with late blowing are *Cl. butyricum*, *Cl. pasteurianum*, *Cl. sporogenes,* and *Cl. tyrobutyricum*. Late blowing may also be attributed to heterofermentative LAB. Sporeforming *Bacillus* spp. such as *B. subtilis, B. pumilus, B. stearothermophilus,* and *B. polymyxa* also can contribute to gassiness in cheese curds and sliminess in brine-treated cheeses.

Yeast and mold contamination and subsequent spoilage is not uncommon in cheeses. Defects include the surface growth of molds, which may produce discoloration and are associated with musty/bitter flavors. Implicated spoilage yeast and molds include *Candida* (black discoloration), *Penicillium* (green discoloration), and *Cladosporium* (green to black discoloration). Cheeses such as cottage cheese and other unripened soft cheeses are susceptible to yeast spoilage during refrigeration. For example, *Torulopsis sphegerica*, *Candida lipolytica*, *Sporobolomyces roseus*, and *Can. valida* have been implicated in spoilage (Fleet, 1990). However, many harder cheeses have waxy coatings or a developed rind which can minimize the spoilage from yeast and mold. Excessive yeast growth can cause softening of cheese due to lipolytic and proteolytic activity.

Yogurt

The Codex Alimentarius (Robinson, 2003) defines yogurt as a milk product obtained by

fermentation by the action of a synbiotic culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. Alternatively, any *Lactobacillus* spp. can be used. Cow's milk yogurt typically has a milk-solids-non-fat of 8.5% to 9% comprised of approximately 4.5% lactose, 3.3%, protein and 0.7% mineral salts. In the first step in yogurt manufacture, the solids content of milk is raised by evaporation under vacuum or, more commonly, by addition of skim milk powder. Ultra-filtration can also be used to achieve this, but its use is rare. The standardized milk is homogenized before being heated to 90°C to 95°C for 5 to 10 minutes or 80°C to 85°C for 30 minutes. After cooling the milk is inoculated with a co-culture of *Strep. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* and either dispensed immediately into cartons and held at 42°C to 43ºC for 3 to 4 hours before refrigeration in the case of set yogurts, or pumped to insulated tanks and held at the same temperature before being filled, usually along with fruit or other flavorings, into cartons and refrigerated to halt any further fermentation.

Yogurt Safety

Salmonella spp., *L. monocytogenes,* and *Campylobacter* spp., along with coliforms, die off and survival of *Staph. aureus, Y. enterocolitica,* and *Aeromonas hydrophila* is questionable. There have been two major food poisoning outbreaks involving yogurt in the United Kingdom. One was due to growth and toxin production by *Cl. botulinum* in a hazelnut flavoring added to yogurt and the other was due to *E. coli* O157:H7, phage type 49, which produced HUS characterized by acute renal failure in some consumers (Morgan et al., 1993).

Yogurt Spoilage

Spoilage is usually due to the growth of yeasts and molds. Examples of the former are *Kluveromyces marxianus* var. *marxianus* and *K. marxianus* var. *lactis, Saccharomyces cerevisiae, Torulopsis* spp., and *Candida* spp., which cause spoilage by producing gas, thus causing the cartons to burst or the foil seals to "dome." Molds grow on the surface; examples of spoilage fungi are species of *Mucor, Rhizopus, Aspergillus, Penicillium,* and *Alternaria* (Robinson, 2003)

Probiotic Cultures

Probiotic organisms have been defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host. They are predominantly comprised of lactic acid bacteria, though not exclusively so (e.g., *Saccharomyces boulardii*). Probiotic strains have mainly been incorporated into yogurts and fermented milk, though there are probiotic cheeses, ice creams, and frozen yogurts. The cultures can act by:

- Competing with pathogenic organisms for limited nutrients
- Inhibiting epithelial invasion by pathogens
- Producing antimicrobial substances
- Stimulating mucosal immunity
- Reducing serum cholesterol, particularly LDL-cholesterol, possibly due to production of hydroxymethyl-glutarate which is reported to inhibit hydroxymethyl-glutarate CoA reductases required for the synthesis of cholesterol
- Aiding in the management of lactose malabsorption
- Preventing rotaviral diarrhea via the phenomenon of competitive exclusion by modifying the glycosylation state of epithelial cells through the action of excreted soluble factors
- Reducing the incidence and severity of *Cl. difficile* diarrhea which can occur as a result of disturbance of the normal gut microflora after antibiotic treatment

- Aiding the prevention and management of allergies which are thought to be due to the delayed colonization of the gut by LAB

For a bacterium to be considered a probiotic, it must (Salminen and Ouwehand, 2003):

- Be nonpathogenic
- Be genetically stable
- Retain viability during manufacture and storage of the product into which it is incorporated
- Survive in the gut
- Possess desirable physiological traits as listed above

Probiotic cultures usually do not grow well in milk and are often used as co-cultures along with *Strep. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus,* which reduce the fermentation time necessary to achieve the required acidity.

Two typical examples of probiotic bacteria are *Bifidobacterium bifidum* and *Lb. acidophilus.*

Bifidobacterium bifidum. *Bifidobacterium bifidum* is isolated from human dental caries, feces, and vagina as well as the intestinal tract of animals. It possesses a metabolic pathway that allows production of acetic acid in addition to lactic acid in the molar ratio 3 : 2. This is important because acetic acid is more bacteriostatic than lactic acid at the same pH value. In addition, the production of organic acids by the organism is thought to stimulate peristalsis and aid normal bowel movement in patients with constipation or irregular bowel movements.

Lactobacillus acidophilus. *Lactobacillus acidophilus* occurs naturally in the mouth, vagina, and gastrointestinal tract of humans and animals. Alone and in combination with *Bifido. bifidum* it has been shown to alleviate the symptoms of patients with irritable bowel syndrome. The organism has also been shown to be effective against *E. coli* O157 : H7 infection by interfering with the transcription of genes involved in colonization and quorum sensing. *Lactobacillus acidophilus* has also been shown to be inhibitory both *in vitro* and *in vivo* to infection with *H. pylori,* the major etiological factor in human gastritis, gastric ulcers, gastric atrophy, and gastric carcinoma.

Prebiotics, which are non-digestible food ingredients and can be oligosaccharides designed with different linkages and different degrees of polymerization, may be used to stimulate the growth of probiotic bacteria in the gut. The combined administration of a prebiotic and probiotic bacterium is termed synbiotic. Prebiotics should neither be hydrolyzed nor absorbed in the gastrointestinal tract, provide a selective substrate to stimulate the growth of probiotic bacteria, and be capable of inducing luminal or systemic effects beneficial to host health.

Concentrated Milk

Concentrated milk can take a number of forms: evaporated, concentrated, condensed, and sweetened condensed milk. Each of these products are dealt with individually in this chapter. More general information can be obtained from Clark (2001) and Robinson and Itsaranuwat (2002).

Evaporated milk differs from the other concentrated milks mainly because of the inclusion of a sterilization stage either before or usually after final packaging. In some countries with low internal milk production, especially in the tropics, evaporated milk is still a general-use milk product.

The Codex Alimentarius standard requires evaporated milk to have at least 7.5% milk fat and 25% total milk solids (Nieuwenhuijse, 2002). Initially the milk can be clarified centrifugally to remove some bacteria, principally heavy spores. In order to stabilize the proteins in the final product, the milk is preheated, usually on a continuous flow basis, at 110°C to 130°C for 1 to 3 minutes to kill vegetative bacteria and some spores. The evaporation or concentration stage is usually

performed at temperatures below 54.5°C using a multistage falling film evaporator; hence the growth of thermophilic bacteria, particularly as biofilms, can occur if the process runs are long and/or cleaning is not adequate. Concentration can be performed using reverse osmosis but this is rare. The concentrate is homogenized and either sterilized and packed aseptically, usually in a can, or subjected to in-container sterilization using temperatures of 115°C for 15 to 20 minutes.

This process should kill all vegetative organisms and spores, although spoilage problems have arisen due to *B. stearothermophilus, B. licheniformis, B. coagulans, B. marcerans,* and *B. subtilis.* If the integrity of the can remains intact and the process has been performed properly, then spoilage is most likely to be the result of the action of heat-stable extracellular enzymes derived from psychrotrophic bacteria growing in the original raw milk.

Concentrated milk, when destined for human consumption as fluid milk, is normally diluted appropriately before use. The raw milk is first pasteurized and then concentrated using the mildest heat treatment possible to minimize undesirable organoleptic changes, then standardized and homogenized before a final pasteurization at an elevated temperature (approximately 79.4°C for 25 seconds) to take account of the slower heat transfer kinetics of the more viscous product. Although the a_w of the product is lower than normal milk, it is not sufficient to inhibit the normal microflora and hence the pattern of spoilage is essentially the same as for pasteurized milk.

Aflatoxin M1 can present a food safety problem when cattle consume moldy grain harboring aflatoxin B1 and it is converted and excreted in the milk by the affected animal.

Condensed milk is used as a source of milk solids for confectionery, bakery products, ice cream, and other processed foods. Condensing is usually in the range of 2.5 : 1 to 4 : 1 depending on its intended use. Initially the raw milk is homogenized and standardized prior to condensing. The treated milk is preheated, usually to 65.6°C to 76.7°C, which can be raised to 82.2°C to 93.3°C to impart different characteristics for particular product applications. The milk is then concentrated in a vacuum pan or multiple effect evaporator, usually at temperatures in the range 54.4°C to 57.2°C. No sterilization process is involved at any stage; therefore, the final product is not sterile and although the a_w is reduced, it is not sufficient to completely inhibit microbial growth. Thus, the product must be refrigerated as quickly as possible and refrigeration maintained during transport to its destination. In general, the process has less lethality than pasteurization and hence the product must not be labeled as pasteurized.

As with evaporated milk, thermophilic bacteria may build up during the condensing stage if the process runs are protracted and the hygiene questionable. However, unlike evaporated milk, there is no subsequent sterilization treatment. In general, the nature of condensed milk emphasizes the need for refrigeration and its rapid incorporation as an ingredient into other more microbiologically stable products. If spoilage occurs it is usually attributed to psychrotrophic bacteria, yeasts, or molds, and is the result of holding the product for protracted periods under improper storage conditions.

Sweetened condensed full-fat milk (SCM) is regulated by the Codex Alimentarius and requires a minimum of 8% milk fat and 28% total milk solids. The product is used for cooking, confectionery including chocolate bars, to enrich tea or coffee, and after dilution, even as a milk drink. The process usually involves forewarming (82°C to 100°C for 10 to 30 minutes), superheating, sugar addition, condensing in a vacuum pan at 57.2°C, cooling, forced crystallization, and finally packaging. Forced crystallization con-

sists of seeding cooled (approximately 30°C) milk with fine lactose crystals to induce formation of numerous small crystals rather than fewer larger ones. Microbial lethality depends on the forewarming stage and superheating phase, if used, while microbial stability of the resultant SCM depends on lowering the a_w and binding available water by the added sugar. The containers used are usually cans, which are treated along with the lids by gas flames, superheated steam, or ultraviolet radiation.

Absence of air in the cans by proper filling inhibits the growth of aerobic microorganisms, particularly molds, yeasts (e.g., *Torulopisis* spp.), and micrococci, which can tolerate high osmotic pressures (although they should have been killed at the forewarming stage). Heat-stable proteolytic and lipolytic enzymes elaborated by psychrotrophic bacteria at the raw milk stage also can cause spoilage.

Dried Milk Powders

Milk powders are produced by the dehydration of liquid milk streams or fractions of dairy streams. Consequently, there is a variety of powders available to the recombined dairy industry and as ingredients to the dairy and other food industries. Among the dried powder products available are: full-cream milk powder, skim-milk powder, whey and whey protein concentrate powders, milk protein/caseinate powders, buttermilk powders, and cream powders. Further tailor-made powder ingredients are commonly produced for specific products, which benefit from functional, textural, and nutritive qualities of the milk-based product. Among the industries using dried milk-based powders are dairy, confectionary, infant formula, and manufactured-food industries, where they are used in coatings, soups, sauces, and ready-to-eat meals.

The key steps in milk powder production are milk clarification, cooling, standardization, evaporation, homogenization (optional), drying, and packaging. From the microbiological standpoint, the quality of the raw milk used and the heat processes applied, evaporation, drying, and prevention of post processing contamination are most important for the microbiological safety of milk powders. Milk streams are given a heat treatment prior to concentration. This preheating eliminates pathogenic bacteria and saprophytic microorganisms and inactivates enzymes such as lipases. Heat treatments are generally 88°C to 95°C for 15 to 30 seconds, although in the separation of whole milk into skim milk and cream, the former may be subjected to defined heat treatments: low heat, 72°C for 15 seconds; medium heat, 75°C for 1 to 3 minutes; and high heat, 85°C for 30 minutes or 90°C for 10 minutes or 120°C to 135°C for 1 to 2 minutes (Augustin and Clarke, 2008).

The milk stream is generally thermally concentrated but may be concentrated through membranes (ultrafiltration or diafiltration). Residence time and temperature exposure are generally less than 60 seconds and lower than 72°C, respectively, during thermal concentration, a process which should further enhance the microbiological quality of the milk powder. In some evaporation plants the milk stream is heated to between 140°C and 150°C, resulting in a product with high microbiological quality. Such products are used as ingredients in baby food manufacture. Spray-drying is the most commonly applied method of drying evaporated milk. Evaporated milk is atomized into fine droplets and exposed to a hot stream in the drying chamber. Milk particles may be heated to 65°C to 75°C during these processes.

Dried Milk Powder Safety

The principal foodborne pathogens associated with dried milk powders are *Salmonella* spp., *Staph. aureus*, *Bacillus* spp., *Cronobacter sakasakai,* and *Clostridium* spp.; outbreaks

related to dried milk have been associated with each of these microorganisms. Numerous cases of salmonellosis were identified in the UK in 1985, mainly affecting infants (46 cases) who had been fed a brand of powdered milk. The implicated serotype, *S. ealing,* was recovered from samples of the product (Rowe et al., 1987; Ryser, 2001). The infection was traced to a malfunctioning spray dryer. A total of 141 confirmed cases under 12 months of age were associated with *S. agona* in a powdered infant formula in France (Brouard et al., 2007). Low levels of salmonellae, not recovered in routine plant sampling, were detected in two formula types produced in the same production line following investigations. This low level of salmonellae in dried-milk-based infant formula is a common feature of such outbreaks as well as rare serotypes being the causative agent. In 2005 an outbreak of salmonellosis was linked to the consumption of contaminated milk powder by hospitalized elderly patients. The incriminating serotype, *S. worthington,* was isolated from environmental samples taken at the manufacturing plant, in milk powder produced in March 2005, and in milk powder produced in December 2004 and stored in the manufacturing plant (Lepoutre et al., 2005). Gastroenteritis infections due to *Salmonella* spp. caused significant morbidity and mortality at three hospitals in Tunisia in 2000. Infective baby powder milk was determined to be the main cause of these infections (Dhiaf et al., 2004).

Investigations recovered injured salmonellae from samples in this study, highlighting the fact that heat-stressed salmonellae may occur in the final product, which makes detection by routine analysis difficult. Licari and Pather (1970) demonstrated that *Salmonella* spp. are not completely eradicated from milk powder by spray drying and are only reduced slightly but not eliminated during storage in milk powder at 25°C and 35°C.

Staphylococcal food poisoning due to ingestion of dried milk powder has been reported in England in 1953, Japan in 1955, Puerto Rico in 1956, and Japan in 2000. In the Puerto Rico outbreak, 775 school children were affected with toxin, demonstrated in the powder, although no *Staph. aureus* cells were recovered (Armijo et al., 1957). *Staphylococcus aureus* enterotoxin (SE) production could take place in raw milk and subsequently survive powder production heat treatments. However, the detection of *Staph. aureus i*n the final product may occur due to a limited survival of the organism during spray drying or is indicative of contamination during manufacture. Whereas the earlier outbreaks associated with dried powder milk occurred in schools and canteens, the most recent outbreak in Japan, in 2000, occurred in a number of households that had used the powdered skim milk as a manufacturing ingredient in causative foods sold at retail outlets (Asao et al., 2003). In the latter outbreak, involving more than 13,000 cases, SE was detected in the end food product and milk powder ingredient. Simulation studies showed that the SE subjected to three heat treatments (130°C for 4 to 5 seconds) in low-fat milk retained immunological and biological activity (Asao et al., 2003), thereby emphasizing the need for good quality raw milk with minimum *Staph. aureus* levels for milk powder production (Soejima et al., 2007).

Cronobacter sakasakii (formerly *Enterobacter sakasakii*) is now recognized as an emerging foodborne pathogen associated with milk-powder-based and other rehydrated powder infant formulas (Farber, 2004; Lehner and Stephan, 2004; Gurtler et al., 2005; Mullane et al., 2007; Van Acker et al., 2001). The outbreaks and sporadic cases have been primarily associated with neonates with a mortality rate of 40% to 80% (Gurtler, 2005). Surveys have shown that the incidence of *Cron. sakasakii* in powdered infant milk formula ranges from 3% in the UK and Ireland (Iversen and Forsythe, 2004; Mullane et al., 2007) to 13% in Southeast

Asia (Mi-Kyoung and Jong-Hyun, 2006) and 25% in the Middle East (Shaker et al., 2007).

Antibiotic-resistant isolates of *Cron. sakasakii* have been detected in dried milk and related products from Egypt (El-Sharoud et al., 2009). These strains could be transmitted from skim milk powder to its related product, imitation recombined cheese, and survive within this product (El-Sharoud et al., 2008). Iversen and Forsythe (2003) have indicated that where *Cron. sakasakii* has been linked to outbreaks, the infection levels have been attained through gross temperature abuse or poor hygiene in the manufacturing process or during preparation of the formula. The principal control measures relevant to *Cron. sakasakii* in infant formula milk powder are control of initial levels in raw milk, reduction of levels during heat treatment of raw milk, prevention of an increase in levels by avoiding post-processing contamination, and provision of appropriate information and preparation instructions to users.

Powdered milk-based infant formulas are heat-treated during preparation but are not commercially sterile. Some reports have suggested relatively high thermal resistance, with this organism surviving pasteurization, while other studies doubt this phenomenon (see Gurtler et al., 2005 for review). The organism appears to have a high tolerance for dessication and can form biofilms, which may help protect it from commercial disinfectants.

Some concerns have been raised about the presence of *B. cereus*, other *Bacillus* spp., and *Cl. botulinum* in dried milk products, especially infant formula. Spores can survive in powders for at least 6 months and Kramer and Gilbert (1989) have cited outbreaks of *Bacillus*-related foodborne illness associated with milk powder and infant formula. Furthermore, toxin-producing *B. subtilis* and *B. lichenformis* strains caused an outbreak of foodborne intoxication in a school nursery in Croatia in 2000 (Pavic et al., 2005).

A public health concern has arisen because pasteurization and spray drying can induce germination and outgrowth of spores. Rapid growth of these organisms could occur in reconstituted milks stored at ambient temperatures. Investigations into the Croatian outbreak revealed that both *Bacillus* strains were able to enter the log phase of growth within two hours of storage of the reconstituted milk at room temperature. Therefore the application of Hazard Analysis and Critical Control Point (HACCP) principles, particularly in relation to reconstitution and consumption of such products, is recommended. Various levels of *B. cereus* contamination in dried whole milk and non-fat dry milk have been reported: United States, 62.5% (Rodriguez and Barrett, 1986); Germany, 13% to 43% (Becker et al., 1994); Germany, 10.1% (Hammer et al., 2001); UK, 17% (Rowan et al., 1997); Brazil, 28% (Costa et al., 2004).

Infant botulism results from the ingestion of spores of *Cl. botulinum*, which germinate, colonize the intestine, and produce neurotoxin *in vivo*. This intoxication is rare; however, an epidemiological investigation of one such case in the UK has advocated a possible link to infant formula milk powder (Brett et al., 2005). *Clostridium botulinum* type B was isolated from an opened container of infant formula from the patient's home and an unopened container of the same batch obtained prior to distribution and retail sale.

Ice Cream

Ice cream can be chemically defined as ice crystals and solidified fat globules embedded in a continuous unfrozen liquid phase comprised of proteins, carbohydrates, salts, and gums. It must contain at least 5% fat and 2.5% milk protein and, in the case of dairy ice cream, the fat component must be exclusively milk fat. One of the main microbiological factors is that ice cream usually contains eggs and ice cream therefore carries the attendant food poisoning risks associated with eggs (e.g., *Salmonella* spp.). Therefore,

pasteurized egg should be used, if added after the heat treatment. In the United Kingdom it is mandatory to heat treat the ice cream mix with one of the following combinations (Papademas, 2002):

- Not less than 65.6°C for not less than 30 minutes
- Not less than 71.7°C for not less than 10 minutes
- Not less than 79.4°C for not less than 15 seconds

After heating, the mix must be cooled to no more than 7.2°C within 90 minutes and stored below −2.2°C, usually around −20°C. During freezing, water crystals form both extracellularly, where they reduce available water for microbial growth, and intracellularly, where they have the potential to perforate the cell membrane.

Challenge testing with pathogens has been performed with ice cream. Certainly *L. monocytogenes* inoculated into both full-fat (10% fat) and reduced-fat ice cream did not significantly decline in numbers during three months of storage at −18°C. In addition, a survey of ice cream in England and Wales by the Public Health Laboratory Service (PHLS) found 2% of ice cream samples to be contaminated with *L. monocytogenes* (Greenwood et al., 1991).

Food poisoning outbreaks have involved ice cream. Perhaps the biggest was caused by *S. enteritidis* in Minnesota, with approximately 22,400 individuals infected (Vought and Tatini, 1998). This was caused by cross-contamination of pasteurized ice cream mix through transportation in a tanker previously used to transport non-pasteurized liquid egg. An outbreak of food poisoning affecting 60 to 80 people due primarily to *Staph. aureus* enterotoxin in Norway was ascribed to ice cream (Kvennejorde, 2003). In Belgium, a food poisoning outbreak due to ice cream occurred as a result of contamination with verocytotoxin *E. coli* serotypes 0145 and 026. The ice cream had been made from pasteurized milk and contamination from one of the food handlers was found to be the most likely cause (de Schrijver et al., 2008).

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Chapter 4

Processing Principles of Dairy Ingredients

Arun Kilara

Introduction

Milk is a highly perishable biological fluid. Its composition and the factors that contribute to variability in the composition have been discussed in Chapter 2. Milk from many farms is collected in tankers two to three times a week and delivered to a processing facility (also known as a dairy plant or factory), where it is stored and processed further to make the appropriate products for which the dairy plant is designed.

Product safety is a major concern in dairy processing. This chapter discusses the regulations for the production and storage of milk at the farm, transportation from the farm to the factory, and the holding and processing on the factory premises. Regulations also apply for standardized food products that must meet compositional requirements as well approved ingredients and processes. In addition, product manufacturers may have internal standards for ensuring the quality of product aspects that are important to the consumer including taste, texture, odor, flavor, mouth feel, color, and keeping quality.

From Farm to Factory

Milk is produced on the farm under strict guidelines that determine its grade (see below). In 2007 the total milk production in the United States was 83.4 billion kg (185.6 billion pounds). Farms with 200 to 500 milk animals accounted for approximately 17.5% of the total milk produced. Farms with 50 to 100 cows and those with more than 2,000 cows accounted for 17.4% and 15% of the total milk production, respectively. Also in 2007, 9.158 million cows were tended by 78,295 production units, which results in an average of 128 cows/farm. The general trend is toward fewer farms with larger herd sizes.

Farms use milking parlors of various designs and the milking interval is unequal. Cows are milked twice/day; however, a small minority milk three times/day. The milk from each animal is weighed and mixed with milk from other animals in the batch of cows being milked. Milk temperature immediately after milking is approximately at the body temperature of the cow (38°C/101°F). Many mesophilic microorganisms can grow at this temperature; therefore, warm milk is cooled rapidly to minimize microbial growth. Cooling is commonly achieved by plate heat exchangers. Milk from several days' milking is collected in insulated tanks called farm bulk milk tanks (Figure 4.1).

Milk collection occurs more frequently on the farm as the number of cows in the herd grows and the numbers of dairy farms shrink. For example, a tanker is dispatched every 45 minutes to Arizona dairy farm milking 7,000 cows twice/day. Smaller farms may use ice bank building tanks. For achieving the best

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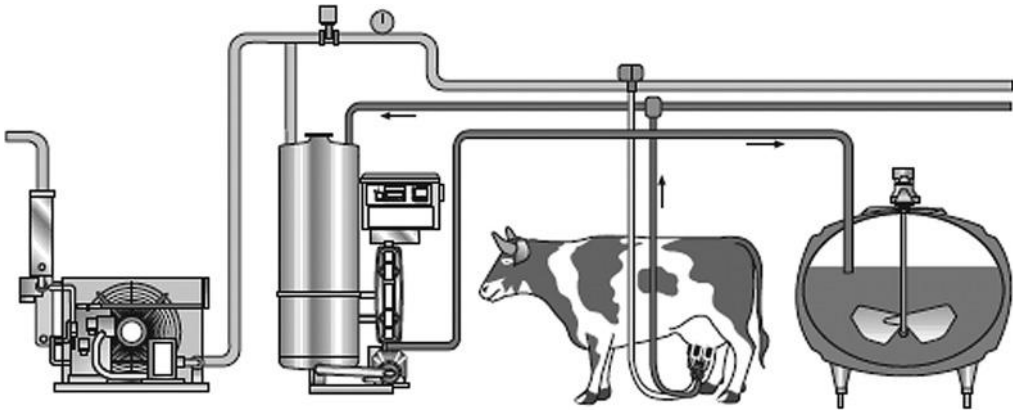

Figure 4.1. Milk from the cow is measured in line and then sent to a bulk cooling tank. Reproduced with permission from Tetra Pak.

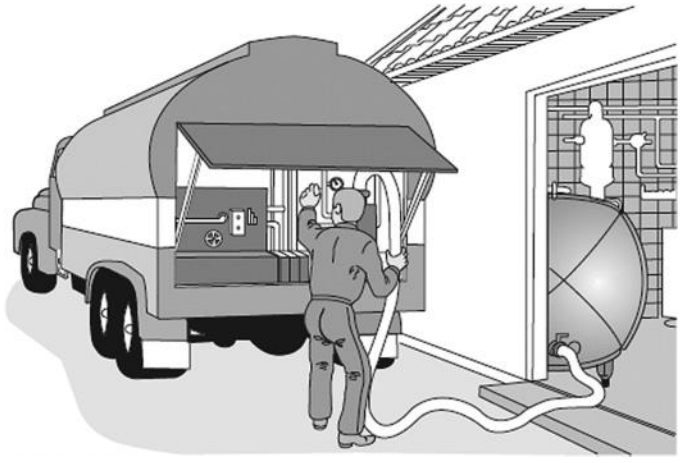

Figure 4.2. Collection of milk on the farm. The tanker pumps milk from the farm bulk milk tank for transport to the dairy factory. Reproduced with permission from Tetra Pak.

grade of milk (Grade A), milk must be cooled to below 4°C (40°F) within time limits, for example, 2 hours post milking.

The tanker driver obtains a sample for milk from each farm at the time of collection. This sample is the basis for quality determination and for payment based on milk composition.

The tanker itself is made of sanitary stainless steel and fitted with baffles to prevent milk from being vigorously shaken during transportation, thus avoiding the possibility of churning the cream into butter. A pump with a volumetric meter and an air eliminating device is located at the back end of the tanker. After the tanker pulls up to the milk shed, the driver attaches a sanitary hose to the farm milk storage tank and pumps the milk into the milk transport tanker (Figure 4.2). When the farm bulk tank is empty the pump is turned off to prevent air from mixing with milk in the tanker. The presence of air can cause foaming and churning of milk. The tanker arrives at the dairy factory after it has collected milk from several farms and is full.

Storage of Raw Milk

The milk tanker enters a covered special reception area at the dairy. A technician from the quality assurance department checks the temperature of the milk and draws a representative sample. The technician also checks the odor of the milk and records whether any off odors are detected. The representative sample collected from each tanker is analyzed for sediments, antibiotic residues, somatic cell count, bacteria count, protein and fat content, and freezing point. Some dairies also may conduct a direct microscopic count of the bacteria present in the milk. The normal bacteria and coliform counts take 24 to 48 hours. The results of the remaining tests are available within 15 to 20 minutes. If all tests meet the standards set by the dairy the milk is unloaded from the tanker.

Sediment tests point to the quality of milk production at the farm. Antibiotic tests indicate whether milk from sick animals was commingled with milk from healthy cows. If such commingling occurs the entire tanker load of milk is rejected. Presence of antibiotics in milk poses a two-fold danger. First, antibiotic-sensitive individuals can suffer from consuming tainted milk. Second, antibiotics may pose a barrier for acidity development in the manufacture of cultured milk products by inhibiting the starter culture growth.

Somatic cell counts are indicative of general animal health. If they are below 500,000/mL of milk the animal herd health is considered good. If the count exceeds 1 million/mL it suggests the presence of mastitis in one or more animals in the heard. Mastitic cows are often treated with antibiotics; the milk from such animals is generally discarded on the farm while the cows are receiving the treatment and for a period afterward.

Protein and fat contents are used to determine payments and to gain full accounting of the raw materials received. This is important for material balance calculations and for determination of losses occurring during processing and packaging. The freezing point of milk test determines adulteration with water, whether accidental or intentional. Adulteration of milk is a prosecutable offence.

The most common procedure is to record the volume of milk delivered by a tanker. Volumetric measurements involve a volumetric flow meter fitted with an air eliminator. Presence of air can distort readings of the volume of milk. The milk passes through the air eliminator and a filter into the metering device prior to going to storage silos. In some dairies, rather than recording the milk volume the tanker may be weighed prior to emptying and after discharging its load.

After discharging its load of milk, the tanker is cleaned in the reception bay or a special cleaning bay. The inside of the tanker is washed by a cleaning-in-place system that rinses the tanker, cleans it with detergents, rinses the detergents, and sanitizes the tanker. The exterior of the tanks also is often washed so that the tankers always look clean on the road. After cleaning and sanitizing the tanker goes to its next round for milk collection.

The raw milk is stored in large vertical tanks known as silos (Figure 4.3). These silos can have capacities of 25,000 to 150,000 liters (6,000 to 37,000 U.S. gallons). The silos are placed outside the dairy with an inside outlet bay. They have double wall construction with an outside welded sheet metal wall and a stainless steel tank within. The silos have methods of agitating milk to prevent gravitational fat separation.

The agitation must be very smooth to avoid rupture of the milk fat globule membranes, which can cause lipolysis of milk fat. Lipolysis generates off flavors and odors. The most common agitation system is a propeller agitator. The tanks contain instruments including a thermometer, level indicator, low level protector, overflow protector, and empty tank indicator. Modern dairies have electronically transmitted data on temperature, levels

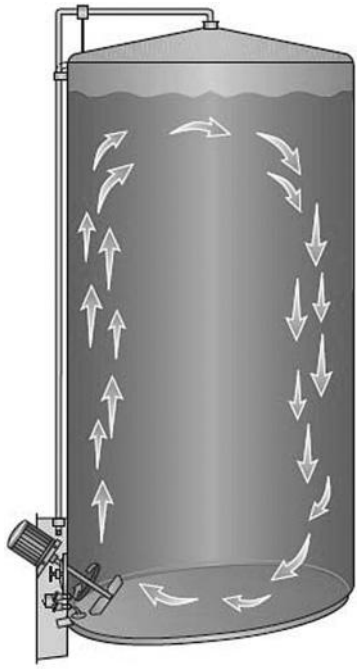

Figure 4.3. Schematic of a milk silo with a propeller agitator. Reproduced with permission from Tetra Pak.

of milk in the silos, and protection devices. Redundant visual (non-electronic) systems may also be employed in some dairies.

Milk storage silos are cleaned in place and periodic visual inspections of the interior surfaces are conducted. Silos are considered confined spaces; therefore, entry into a silo is strictly according to the standards recommended by Occupational Safety and Health Administration of the U.S. government.

The temperature of the milk in the silo must be maintained at 4°C or below (below 40°F). Even at these temperatures, psychrotrophs can cause proteolysis and lipolysis if milk is stored for long periods. Therefore, it is recommended that the silos be regularly emptied, cleaned, and sanitized. The raw milk in the silo is further processed; the main elements in the processing are centrifugal operations, thermal treatment, homogenization, cooling, and packaging.

The processing steps may involve one or more operations in combination, and the most common operations involve pumping or transferring fluids, heat transfer (cooling and heating), mixing ingredients, separation (fat standardization), and microbial transformation of milk (acid gel formation). These aspects are discussed below.

Overview of Processing Equipment in a Dairy Plant

Fluid Transfer Operations

Fluid transfer processes involve using pumps to transfer milk from the receiving tankers to storage silos and then to appropriate unit operations. The two main categories of pumps used in the dairy industry are centrifugal and positive displacement pumps. There are different types of pumps within each category.

The selection of the right type of pump for use in an operation depends upon a number of factors including flow rate, product to be handled by the pump, viscosity, density, temperature, and pressure in the system. Pumps should be installed as close to the tanks and with as few valves and bends in the line as feasible. Any devices to restrict flow should be placed at the exit or discharge side of the pump. Cavitation is a pumping problem that is caused by too low of pressure at the inlet end of a pump relative to the vapor pressure of the fluid being transferred. As cavitation progresses, pumping efficiencies decrease and eventually the pump ceases to transfer the fluid. The appropriate size of the pump required for the transfer depends upon flow rate and head, required motor power, and the net positive suction head. Engineers using charts and formulas easily calculate these parameters.

Centrifugal Pumps

In a centrifugal pump, a motor drives an impeller with vanes (Figure 4.4). The motion is circular and the liquid being pumped enters to the center of the impeller, which imparts a

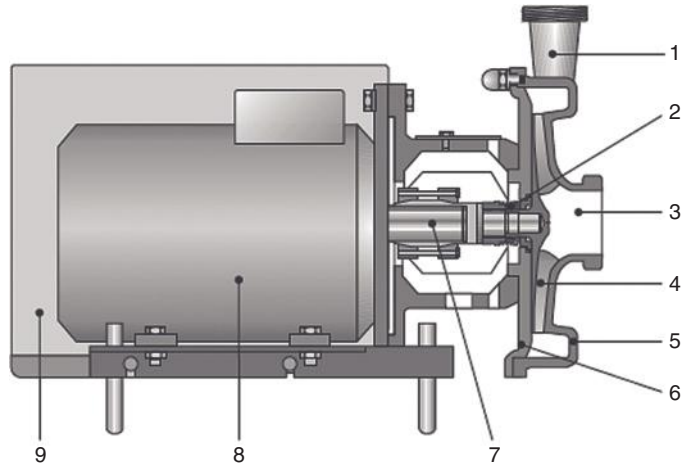

Figure 4.4. Centrifugal pump showing (1) delivery line, (2) shaft seal, (3) suction line, (4) impeller, (5) pump casing, (6) back plate, (7) motor shaft, (8) motor, (9) stainless steel shroud and sound insulation. Reproduced with permission from Tetra Pak.

circular motion to the liquid. The liquid exits the pump at a higher pressure than that at the inlet. Centrifugal pumps are useful for transferring liquids that are not very viscous. These pumps are widely used in most applications in a dairy factory due to their lower costs (compared with positive displacement pumps). Centrifugal pumps are not suitable for high viscosity liquids or those products that require care in handling, for example, fluids in which structures should not be disturbed or ingredients whose identity is critical to product appeal. Flow control is achieved by three different means. Throttling is expensive but offers the greatest flexibility. Changing the impeller diameter that is the most economical but the least flexible. Installing an electronic speed controller is both economical and provides flexibility.

Positive Displacement Pumps

Positive displacement pumps work on the principle of positive displacement in which each rotation or reciprocating movement results in a finite amount of fluid is being pumped, regardless of the manometric head. The main types of positive displacement pumps are rotary and reciprocating pumps. They are useful for higher viscosity fluids; at lower viscosities they may exhibit some slip as the pressure increases. The net result is a reduction in volumetric flow on each stroke. Throttling by flow control valves at the discharge end of the pump should be avoided, and these pumps must be fitted with a pressure relief valve. Flow control in positive displacement pumps is achieved by controlling the speed of the motor or adjusting the volume of reciprocating pumps. Positive displacement pumps must be placed as close to the feed tank as possible and the dimensions of the pipes should be large relative to those of centrifugal pumps. If pipe diameters are too small, the pressure drop may be high enough to cause cavitation in the pump.

Positive lobe pumps generally have two rotors, with three lobes on each rotor (Figure 4.5). A vacuum is created when the lobes move, causing the process fluid to fill the cavities of the lobes. The process fluid is then moved along the outer walls of the pump toward the discharge end. The rotors are driven independently by reducing gear motors. The lobes do not touch each other or the walls of the pump casing. These pumps are used when the viscosity of the process fluid exceeds 300 cP, such as when

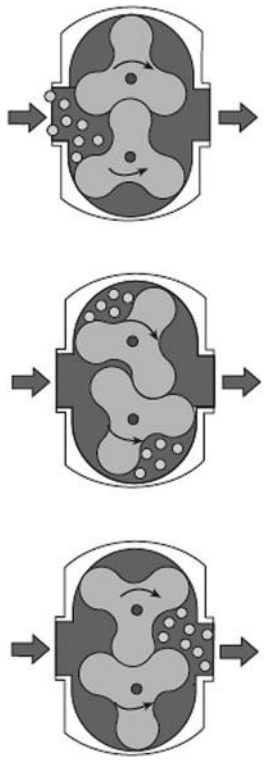

Figure 4.5. Lobe rotor principle. Reproduced with permission from Tetra Pak.

transferring cream and cultured products. Eccentric screw, piston, and diaphragm pumps are also positive displacement pumps that are used for specialized purposes in dairy plants.

Heat Transfer Operations

Heating and cooling are two common operations in any dairy plant. Collectively these operations involve the transfer of heat from one medium to another. Transfer of heat can be routinely achieved through indirect contact of a hot medium against a cool medium. In the case of heating dairy fluids, the hot medium is hot water. Boilers produce steam that is directly injected into the water and the result is hot water. In the case of cooling, a cold medium removes heat from a dairy fluid. This cool medium may be incoming cold raw milk (as is the case of the regeneration section of a pasteurizer) or chilled water. Chilled water is produced by contacting water with a refrigerant (commonly ammonia in the United States). The apparatus in which heating or cooling takes place is generically called a heat exchanger.

Calculating the heat transfer area required for a particular operation is a complex process involving product flow rate, physical properties of the fluid being heated and the heating medium, the temperature program necessary for the operation, allowed pressure drops, design of the heat exchanger, sanitary requirements, and necessary operational time. The product flow rate depends on the operating capacity of the dairy factory.

Density and specific heat and viscosity are important parameters that define the physical properties of the fluids. The temperature program depends on the legal requirements and temperature differentials between the medium being heated and the heating medium. Temperature changes (often referred as Δt) depend upon the inlet temperatures of the medium being heated and the heating medium. The design of the heat exchanger includes the flow of the fluid being heated in relation to the flow of the heating medium. Such flows can be countercurrent (Figure 4.6) or concurrent (Figure 4.7), meaning the fluid being heated flows against the flow of the heating medium or in the same direction as the heating medium, respectively. The design also includes the physical nature of the heating apparatus, including plate heat exchangers or tubular heat exchangers, and in some cases scraped surface heat exchangers.

The ability to effectively clean and sanitize food contact surfaces is vital in the food industry and therefore the design of a heat exchanger must take this into consideration. The necessary operational time is the length of time the equipment can be operated without cleaning, and it depends on a number

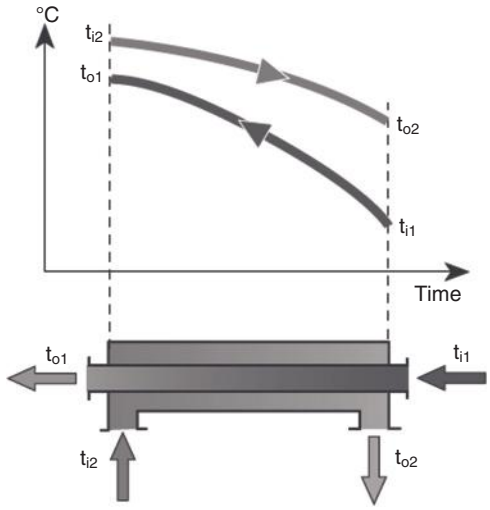

Figure 4.6. Temperature profile for a product in a countercurrent heat exchanger. Red line/fill, heating medium; blue line/fill, product flow; t_i, inlet temperature; t_o, outlet temperature; subscripts 1 and 2, product and heating medium, respectively. Reproduced with permission from Tetra Pak.

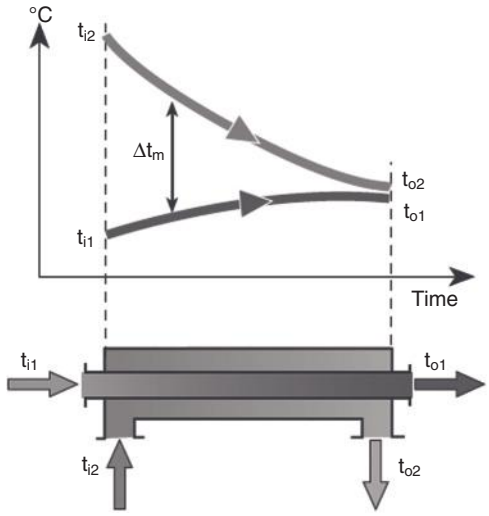

Figure 4.7. Heat transfer in a concurrent heat exchanger. Red line/fill, heating medium; blue line/fill, product; T_i, inlet temperature; t_o, outlet temperature; subscripts 1 and 2, product and heating medium, respectively. Reproduced with permission from Tetra Pak.

of factors. The operational time cannot be predicted and varies from factory to factory.

Refrigeration is another aspect of cooling; it involves the removal of heat from a product, a process in which the product cools down and the medium removing the heat warms up. In the dairy industry, refrigeration is commonly achieved by chilled water or polyethylene glycol in some cases. The water is chilled by contacting it with a refrigerant such as ammonia or other fluorocarbon gases.

Mixing Operations

The manufacture of many dairy products involves mixing ingredients into milk. For example, in the manufacture of flavored milks, sweeteners, stabilizers, and flavorings are added to milk prior to processing. To fortify solids in certain types of yogurts, milk solids are added to milk prior to pasteurization. In other instances, storage of raw milk in silos necessitates periodic agitation of the contents of the silo. In batch pasteurization, the milk is heated in a tank with an agitation system to ensure uniform heat transfer. In all these instances, mixing is required and is achieved by a number of means.

Batch and continuous processes are available to incorporate solid ingredients into milk. The simplest batch blending system is a funnel or hopper to feed the dry material to a closed-circuit circulation of the process fluid. A centrifugal pump is used to circulate the process fluid after the tank is filled (Figure 4.8). The centrifugal pump can be placed at either the suction or discharge sides of the hopper. If the hopper is on the suction side of the pump, powders are rapidly and efficiently dispersed as the mixture of powder and fluid come in contact with the impeller of the pump. The disadvantage is that frequent blockages may occur in the hopper. Placing the hopper on the discharge end of the pump avoids the blockage problem. This configuration requires a venturi to facilitate the mixing (Figure 4.9).

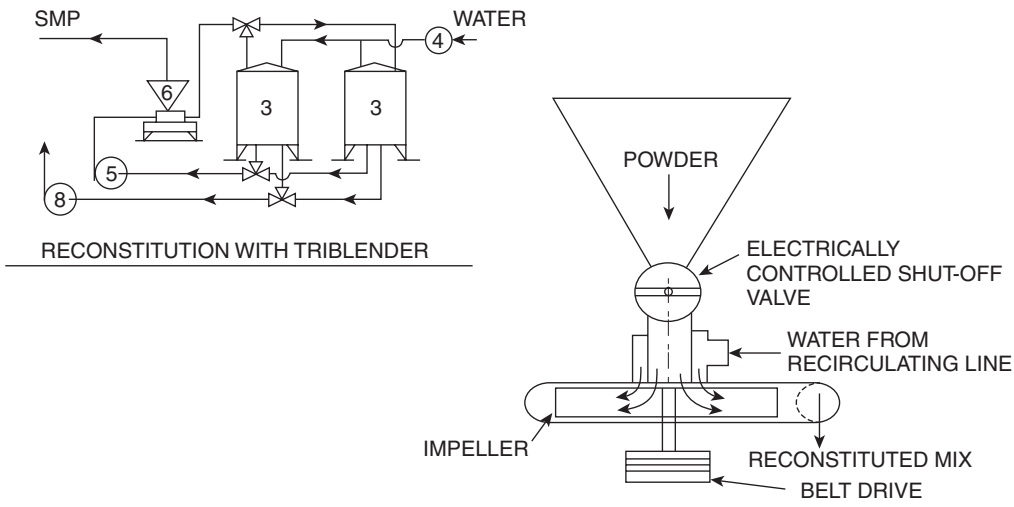

Figure 4.8. Mixing dry ingredients using a triblender.

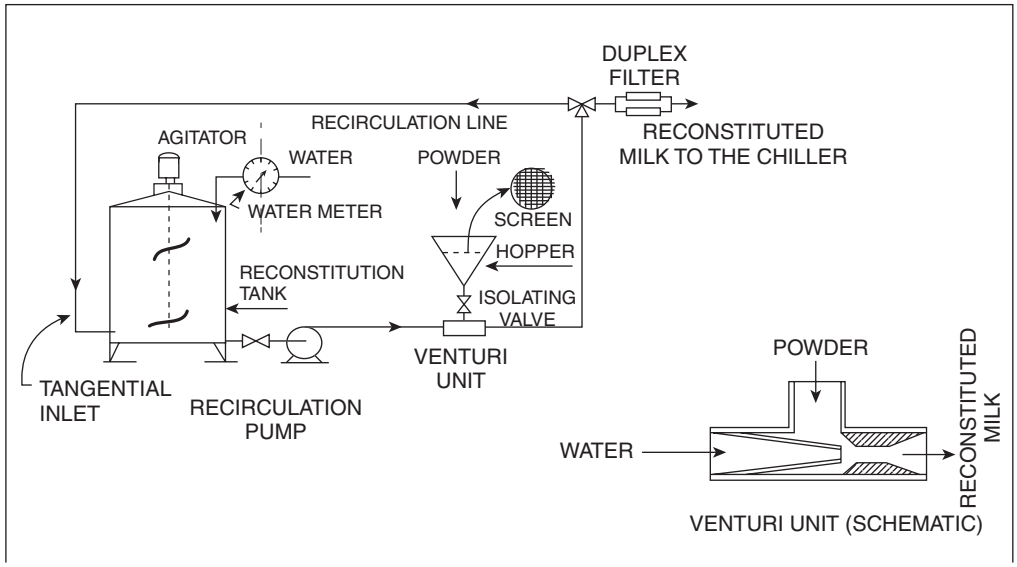

Figure 4.9. Reconstitution in a system with a venturi; the dry ingredients are added at the discharge side of the pump.

Another type of batch mixing occurs in tanks and silos. The tanks are equipped with paddle, propeller, or scraped surface agitators. The agitators can be positioned at the top or bottom, perpendicular, or centrally mounted. In addition to the type and placement, the speed of agitation, tank geometry, vortex creation, air incorporation, and shearing effects impact the mixing efficiency.

Many types continuous mixing systems, also called in-line mixers, are available. In devices such as the Tri-Blender and Breddo

Likwifier, a high-speed blender, the powder and process liquid are contacted and sheared in the mixer. Silverson, another in-line mixer, operates at high speeds and its action is somewhat similar to homogenization.

Separation

It is necessary to separate the fat from the milk. The principles used to separate fat from milk are also applied to remove fine extraneous material from milk and to reduce the bacterial content of milk. Separation of fat from milk is called cream separation, the removal fine extraneous particle is termed clarification, and the reduction in microbial numbers is obtained through bactofugation. All of these processes rely on centrifugal force to achieve their objective. The factors that affect the efficiencies of these processes are diameter of the particles (d μ), density of the particle (ρ_p kg/m^3), density of the continuous phase (ρ_l kg m^3),viscosity of the continuous phase (η kg/ms) and the gravitational force (g = 9.81 ms^2). For example a 3 μ diameter fat globule will rise at a velocity of 0.6 mm/h. To speed up this process centrifugal force is applied and the sedimentation velocity is increased 6,500-fold. Specially designed equipment called a cream separator is used to achieve this separation under a centrifugal force field.

Another centrifugal operation in the dairy industry is a variant of cream separation that is used to remove solid impurities from milk. This piece of equipment is called a clarifier. The principal difference between clarification and separation is in the design of the disc stack in the centrifuge bowl and the number of outlets. In a clarifier, the disc stack has no distribution holes and only one outlet. In a separator disc there are distribution holes and are two outlets, one each for cream and skim milk.

Bactofugation is a third application of centrifugal force in dairy processing. In this process centrifugal force is used to reduce the bacterial content of milk. Sporeformers are effectively reduced by this process, which is more commonly used in treating milk for powder and cheese manufacture.

Microbial Transformation

Drying, condensing, and fermentation are all methods of preserving milk. Fermentation is the controlled acidification of milk and cream, in which the type of microorganisms growing and the conditions for their growth are carefully monitored and stopped. The characteristics of the microorganisms used in fermenting milk and cream are discussed in greater detail in Chapter 6. The main concepts of this transformation are outlined below.

Lactic acid bacteria are the prime agents of fermentation. Morphologically these are rods and cocci and they stain Gram-positive. The optimal temperatures for their growth are either in the mesophilic range (20°C to 30°C; 68°F to 86°F) or thermophilic range (35°C to 45°C; 95°F to 113°F). Lactic acid bacteria use lactose to produce lactic acid. The transport of lactose into the cells is facilitated by two enzyme systems: the phosphoenol pyruvate dependent phosphotransferase system and an ATPase dependent system. Lactic acid bacteria are also classified as homofermentative or hetrofermentative. Production of lactic acid only from lactose, as is the case with most mesophilic lactic acid bacteria, leads to such bacteria being labeled homofermentative. One molecule of lactose results in four molecules of lactic acid. Hetrofermentative lactic acid bacteria, including leuconostocs, lack the enzymes called aldolases and cannot ferment lactose via the glycolytic pathway. This class of bacteria ferments one molecule of lactose to two molecules each of lactic acid, ethanol, and carbon dioxide. Homofermentative lactic acid bacteria do not produce ethanol or carbon dioxide, whereas hetrofermentative lactic acid bacteria do.

In addition to lactic acid being produced during fermentation, caseins are also being modified by proteolytic enzymes. Other changes occurring in milk may produce polysaccharides which can alter the viscosity of the milk. Some lactic acid bacteria metabolize citric acid to produce aroma volatiles such as diacetyl.

Milk fermentation is necessary for the manufacture of yogurt, buttermilk, kefir, and cheeses, whereas the fermentation of cream is essential for the manufacture of sour cream, cream cheese and other types of cheeses, and cultured cream butter. Some of these aspects are discussed in greater detail in Chapters 6, 11, 16, 17, and 18). Common milk processing steps are discussed below.

Centrifugal Operations

Centrifugal operations remove some or most of the fat, a step called standardization. One method is to completely remove all of the fat as cream, leaving skim milk. The cream and skim milk can then be recombined in desired ratios to obtain low-fat, light, and whole milk with 1%, 2% and 3.25% fat, respectively. This standardization usually is performed in a continuous manner.

Cream is separated from milk in a cream separator. Often the separator has the ability to remove sediment from milk as well as separate the cream from milk. Depending on the design of the separator-clarifier, the sediment collected can be manually or automatically removed. Typically, milk can have 1 kg of sediment/10,000 liters (1 lb/1,100 U.S. gallons). Automatic discharging separator-clarifiers are hermetically sealed and cleanable in place. This is less cumbersome than opening up the bowl assembly and manually cleaning both the sediment and disc stacks of a separator.

Fat content in cream is controlled by a paring disc in conjunction with a cream flow meter. A throttle valve at the cream discharge controls the volume of cream leaving the separator. This is counterbalanced by controlling the pressure of skim outlet and depends on the make and throughput of the separator.

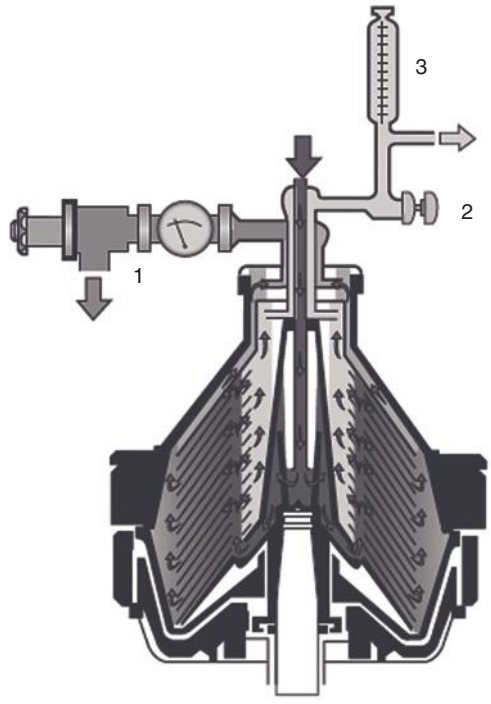

Figure 4.10. Paring disc separator with manual controls. 1, skim milk outlet with regulator; 2, cream throttling valve; 3, cream flow meter. Reproduced with permission from Tetra Pak.

In paring disc separators the volume of cream discharged is controlled by a cream valve with a built-in flow meter (Figure 4.10). The size of the valve aperture is controlled by a screw and the throttled flow passes through a graduated glass tube with an indicating device. Balancing the cream flow and the skim milk pressure produces the desired fat content in the cream.

In the more common hermetically sealed separators, milk is supplied to the bowl through the bowl spindle. It is accelerated to the same speed as the rotation of the bowl and continues through the distribution holes in the disc stack. The bowl of a hermetic separator is completely filled with milk

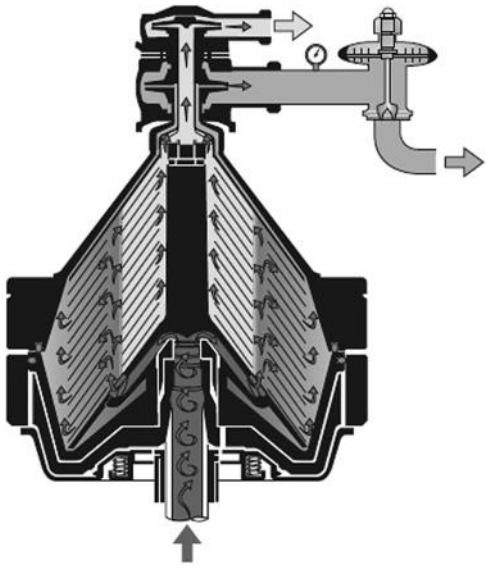

Figure 4.11. Hermetic separator bowl with an automatic pressure unit on the skim milk outlet. Reproduced with permission from Tetra Pak.

during operation. There is no air in the center, hence the name hermetic separator. It is a part of the closed piping system of the dairy. The pressure generated by the external product pump is sufficient to overcome the resistance to flow through the separator to the discharge pump at the cream and skim outlets.

An automatic constant pressure unit in a hermetic separator is controlled by a diaphragm valve. The pressure on the valve is controlled by compressed air above the diaphragm (Figure 4.11).

Direct in-line standardization of fat content of milk is based on the principle of keeping the pressure of the skim milk constant. This pressure must be maintained regardless of flow fluctuations or pressure drop caused by the equipment after separation. Pressure is maintained by a constant pressure valve at the skim discharge side of the separator. Precision standardization also depends upon fluctuations in fat content of the incoming milk, throughput, and preheating temperatures.

Some countries may use centrifugal operations to manufacture cultured dairy products. In yogurt manufacture, skim, 1%, and 3.25% milk is often used and in the more indulgent types of yogurt higher fat contents up to 8% may be used. All of these different fat contents are obtained through centrifugal operations involving standardization on line. A schematic of an in-line standardization unit is shown in Figure 4.12.

Separation temperature is also an important variable. Cold separation of milk (below 4°C or 40°F) decreases the efficiency of fat recovery. Therefore, warm separation is commonly used where the efficiency of fat removal is greater because the fat is in a fluid state at temperatures of around 50°C (122°F). Warming the milk can take place during the regeneration phase of heat transfer (see below).

Thermal Processing Systems

The standardized milk is thermally processed, or pasteurized, as required by law to render the milk free from pathogens. Pasteurization can be a batch process or a continuous process. Batch processes are used by small processors; they are uncommon in modern dairies. The batch process is called long-time-low-temperature (LTLT) pasteurization.

In the batch process, standardized milk is heated to 62.5°C (145°F) and held at that temperature for 30 minutes. The processing tanks used for such purposes should have the characteristics defined in the pasteurized milk ordinance (PMO). Homogenization takes place post pasteurization and is followed by cooling. Homogenization may also take place after the regeneration section and prior to entering the heating section. If the temperature of the milk is around 40°C (104°F), lipolysis can be enhanced by homogenization. Therefore, homogenization temperature must be above 45°C (113°F). At this temperature milk lipase and many microbial lipases are rendered ineffective.

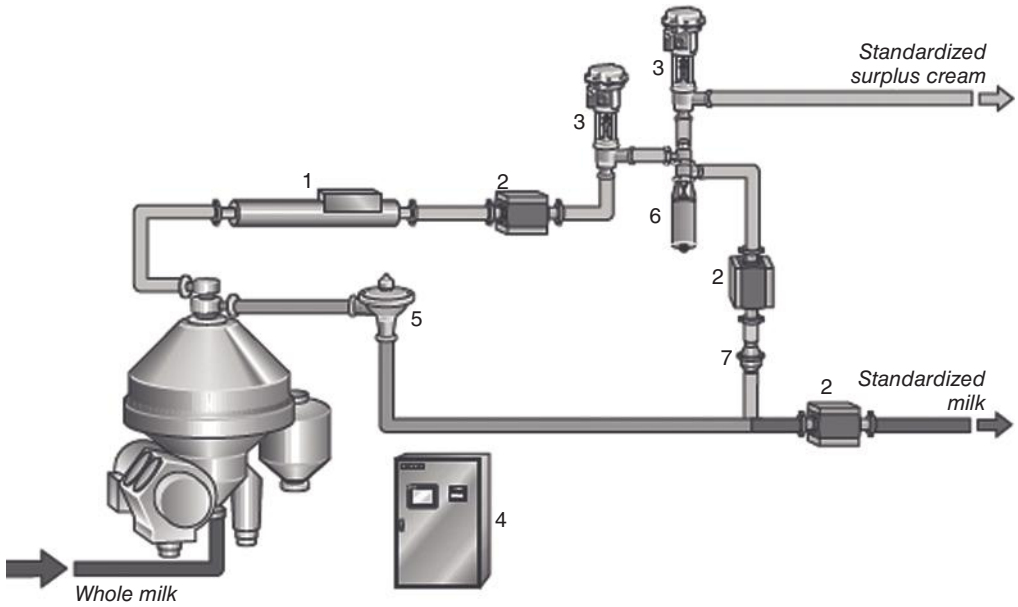

Figure 4.12. The complete process for in-line standardization of milk and cream. 1, density transmitter; 2, flow transmitter; 3, control valve; 4, control panel; 5, constant pressure valve; 6, shut-off valve; 7, check valve. Reproduced with permission from Tetra Pak.

The continuous pasteurization process is known as high-temperature-short-time (HTST) pasteurization and entails heating milk to 71.5°C (161°F) and holding the milk for a minimum of 15 seconds prior to cooling and storage. Yogurt manufacture necessitates the holding of milk for longer periods of time to denature the whey proteins and thus improve the gel strength of yogurt. Therefore, in yogurt manufacture milk may be held at 71°C for 30 minutes or it may be heated to 90°C (194°F) and held for 10 minutes. The HTST process involves plate heat exchangers; the PMO has prescribed various controls and requirements for the equipment.

The effect of heat treatment on milk is to reduce the rate of deterioration due to microbial and enzymatic action. In addition, the milk may look whiter, appear more viscous, and have appreciable flavor changes and a decrease in nutritive value. The effectiveness of pasteurization is estimated by assaying for the enzyme phosphatase. No phosphatase activity is detected in fresh properly pasteurized milk. Sometimes microbial phosphatases or the milk phosphatase itself can regain some of its activity during storage. If the presence of phosphatase is detected in stored pasteurized milk, further tests are often conducted to determine the cause of this positive test.

In the HTST pasteurization process (Figure 4.13), cold milk enters a balance tank with a float valve. The balance tank (also known as a constant level tank) maintains a constant level of milk in the plate heat exchanger, because the pasteurizer should be filled at all times during operation to prevent the product from burning onto the plates. The balance tank may be fitted with an electronic sensor that transmits a signal to the flow diversion valve. If the level in the balance tank drops below a certain level and fresh milk is does not come in to make the level up, this electrode transmits a signal for the flow diversion valve to open and to return the

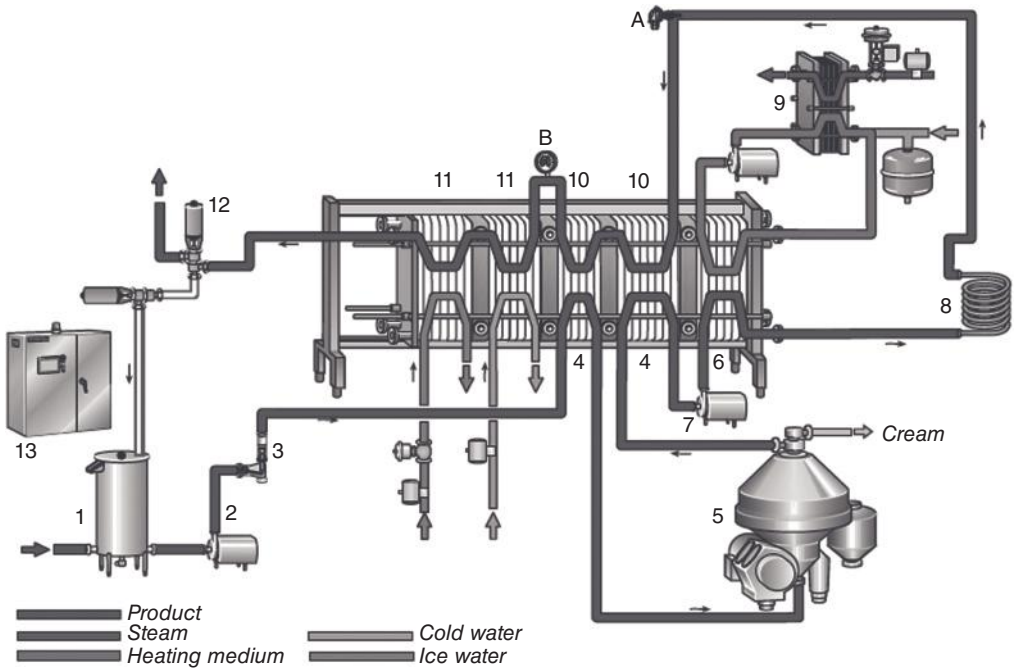

Figure 4.13. A complete pasteurizer plant. 1, balance tank; 2, feed pump; 3, flow controller; 4, regenerative pre-heating sections; 5, centrifugal clarifier; 6, heating section; 7, booster pump; 8, holding tube; 9, hot water heating; 10, regenerative cooling sections; 11, cooling sections; 12, flow diversion valve; 13, control panel; A, temperature transmitter; B, pressure gauge. Reproduced with permission from Tetra Pak.

milk in the system to the balance tank. The milk is replaced by water if circulation has continued for a certain predetermined time.

Milk is pumped from the balance tank to the plate heat exchanger. The pump is fitted with a flow controller to ensure that a constant flow is maintained at a predetermined value. This value depends on the characteristics of the pump and the heat exchanger capacity. The flow control device also guarantees a stable temperature and constant length of holding. The flow control device also may be located after the first regeneration section.

Regenerative preheating is an energy-saving step in pasteurization. Cold untreated milk is heated by the outgoing pasteurized milk. Thus, cold milk is preheated and the hot milk is cooled simultaneously. The regeneration section is divided into two sections. After the cold milk is preheated in the first regeneration section, it can be separated and homogenized and then the standardized, homogenized milk enters the second regeneration section, where it is further heated by the hot pasteurized milk. Heating is accomplished by using hot water as the medium. The hot water, in turn, is produced by injecting culinary steam into the water. The steam is generated in boilers at the dairy factory.

After the regeneration section the milk enters the pasteurization section where it is heated to the required temperature. The heated milk exits the heating section and enters an external holding tube. The flow rate of hot milk determines the residence time in the holding tube. The flow rate in turn is controlled by the flow controller that was referred to earlier. After the transit through the holding tube the exiting milk temperature is measured and transmitted to a temperature controller and a recording chart.

A sensor at the exit of the holding tube transmits a signal to the temperature monitor. As soon as the temperature falls below a preset minimum value the monitor switches the flow diversion valve to diverted flow. In diverted flow, the hot milk returns to the balance tank because it is not considered pasteurized. The reason for the fluctuation is determined and corrected and if the correct temperature is maintained at the exit point of milk from the holding tube, further flow is continued past the flow diversion valve. Often a booster pump may be added after the milk exits the holding tube. The hot pasteurized milk enters the regeneration section of the pasteurizer to heat the incoming raw milk.

In the regeneration section unpasteurized milk flows on one side of the plate and hot pasteurized milk flows on the other. If there are pin holes in the plates of the heat exchanger, unpasteurized milk can commingle with pasteurized milk. This violates the integrity of the pasteurized milk and the fluid is not considered pasteurized. To avoid such a problem, the pasteurized milk is always at a higher pressure than the raw milk. A pressure differential meter is often installed on the control panel to measure the pressures. If the pressure differential between raw and pasteurized milk drops below a preset value, a signal is sent to the flow diversion valve to open. Thus, the two different causes for flow diversion are temperature falling below preset values and the pressure differential between raw and cold milk falling below a certain preset limit. The milk is not considered pasteurized if either of these events occurs. For milk to be designated as pasteurized, every drop must be heated to and held at the specified minimum temperature for a specified amount of time.

Pasteurized milk in the regeneration section is cooled, giving off its heat to the cold incoming raw milk. This cools down the milk but not to the desired 4°C (40°F) or below. The final step in pasteurization is to cool the milk to below 4°C in the cooling section. Cooling is achieved by chilled water or cold glycol as the refrigerant. The water is chilled by a refrigeration system which commonly uses ammonia as the refrigerant. Other hydrocarbons also may act as refrigerants. Because the pasteurized milk transmits considerable heat to the cold raw milk, less refrigeration capacity is required to cool the milk to below 4°C.

In yogurt manufacture the cooling system may not be used. Once the pasteurized milk has been cooled to around 43°C to 45°C it may be pumped to the fermentation tanks for further processing. Obviously, reheating cold pasteurized milk to the incubating temperatures of 43°C to 45°C requires more energy than avoiding this step in the first place.

Homogenization

Homogenization reduces the size of fat globules to prevent creaming (separation of a fat-enriched layer from the aqueous phase). The globule size is reduced through a combination of turbulence and cavitation in a homogenizer.

Cold milk cannot be homogenized efficiently because the milk fat is still solid. Therefore, homogenization occurs best at temperatures greater than 37°C (99°F). Another necessity for efficient homogenization is the presence of protein. A minimum value of 0.2 grams of casein/gram of fat is recommended.

Homogenizers are manufactured as single-stage and dual-stage machines. In single-stage homogenization the whole pressure drop is used over one device. It is used for products with low fat content and in products requiring a high viscosity (e.g., sour cream, coffee cream, whipping cream). Two-stage homogenizers are used in breaking down the fat globule in two stages. This is effective for high fat content, high solids content, or products in which low viscosity is desired (Figure 4.14).

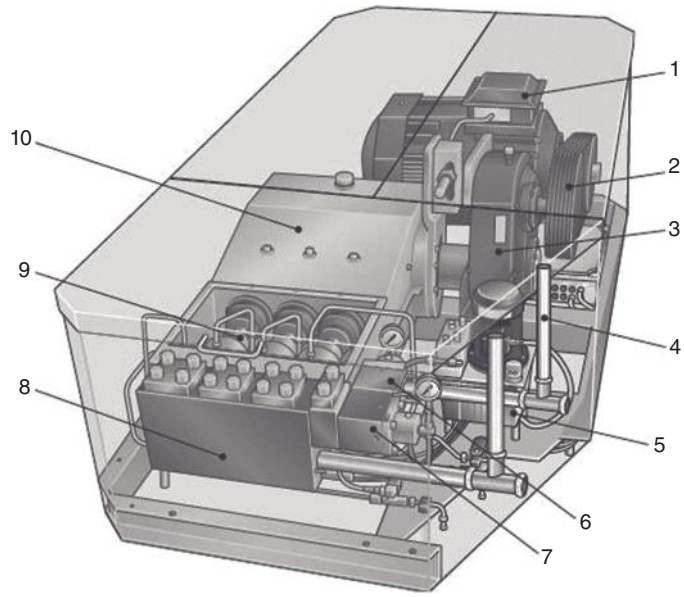

Figure 4.14. The homogenizer is a large, high-pressure pump with a homogenizing device. 1, main drive motor; 2, V-belt transmission; 3, gear box; 4, damper; 5, hydraulic pressure setting system; 6, homogenizing device 2nd stage; 7, homogenizing device 1st stage; 8, solid stainless steel pump block; 9, pistons; 10, crankcase. Reproduced with permission from Tetra Pak.

The results of homogenization are smaller fat globule size (prevention of creaming), whiter and more appetizing color, reduced sensitivity to fat oxidation, and a fuller bodied flavor and mouth feel. In cultured milk products a better stability is also achieved. Homogenizers are high-pressure machines in which reciprocating pistons create the pressure. Pressurized milk is passed through a narrow aperture. When the pressurized milk exits into atmospheric pressure, cavitation is created which results in large fat globules being reduced to smaller ones. The narrow aperture is called the homogenizer valve. There are many designs for the homogenizer valve, all of which have a similar effect on the fat globule (Figure 4.15).

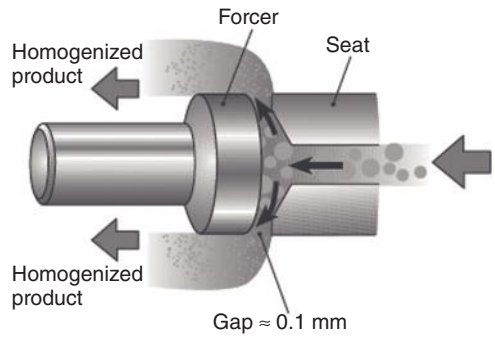

Figure 4.15. The milk is forced through a narrow gap which results in the fat globules splitting into smaller sized droplets. Reproduced with permission from Tetra Pak

When a large fat globule is disintegrated to a number of small droplets, a tremendous increase in surface area of the fat occurs. Onto the surfaces of these newly created droplets casein adsorbs and stabilizes the droplet. If this step does not occur, the fat droplets could recombine to form a larger globule. The adsorption time has been estimated to be around 0.25 μ, the encounter time between the protein and fat is estimated to be 015 μ, and the deformation time is around 0.3 μ for 4% fat milk being homogenized at

20 MPa. In this process, 4% milk, which has an average fat globule diameter of 9 μ, is reduced to 1.6 μ. The protein that is adsorbed onto the newly formed surfaces is casein. Approximately 75% of the surface area is covered with casein. Larger micelles are preferentially adsorbed over smaller ones. Protein adsorption is greatest on smaller globules. The surface concentration of protein has been measured at 10 mg.m^2.

Membrane Technology

Membrane technology is useful for selectively enriching certain components. Membrane technology consists of four distinct processes: Reverse osmosis (RO) concentrates solids by removing water. Nanofiltration (NF) can concentrate organic components by removing monovalent ions such as sodium and chloride, thereby resulting in demineralization. Ultrafiltration (UF) is the process in which macromolecules are concentrated; the major macromolecules in milk are fat and proteins. Microfiltration (MF) removes bacteria and can separate macromolecules.

These techniques use a cross flow membrane in which the feed solution is forced through the membrane under pressure Figure 4.16). The solution flows over the membrane and solids are retained (retentate) while the removed materials are present in the permeate. The membranes are classified according to their molecular weight cutoff, supposedly

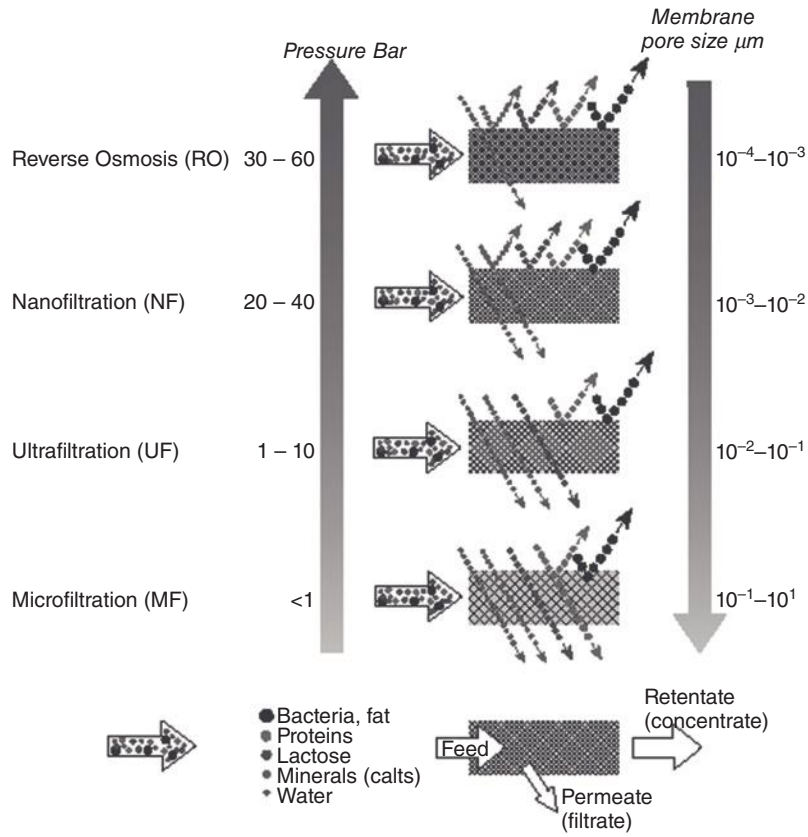

Figure 4.16. Different membrane processes and their characteristics. Reproduced with permission from Tetra Pak.

the molecular weight of the smallest molecule that cannot pass through the pores of the membranes.

The filter modules themselves are available in various geometries. The most common are spiral wound; others are plate and frame, tubular, and hollow fiber. Tubular filters can be made out of ceramics or polymers.

Membrane separation capacity depends on a number of factors. Foremost is membrane resistance, which is determined by membrane thickness, surface area, and pore diameter. Next is transport resistance (also known as fouling effect), which occurs on the membrane surface as filtration proceeds. The formation of a layer of deposit leading eventually to membrane fouling is due to the flow of macromolecules at right angles to the direction of flow. A concentration gradient leads to diffusion in the opposite direction. Parallel to the membrane, the macromolecules present in the layer close to the membrane move at varying velocities depending on the axial flow rate. The concentration polarization is not uniformly distributed, especially when the pressure drop gives different transmembrane pressures along the membrane surface. The upstream end of the membrane clogs first and gradually spreads across the whole surface of the membrane, reducing capacity and making cleaning necessary.

Membrane operations can be batch or continuous. Continuous processes are more desirable in dairy plants. Process temperatures are maintained at around 50°C (122°F) to minimize microbial growth and to improve membrane flux.

The use of membrane processing in the cultured dairy products area is restricted to concentration of skim milk for fat-free yogurt manufacture. Some of the lactose and minerals are removed from skim milk, thereby increasing the protein content. This process can concentrate skim milk with 9% solids to 12% solids. There is still enough lactose in the retentate to facilitate fermentation. A higher protein content in the concentrated milk results in a firmer acid gel in yogurt. Membrane processes are extensively used in the manufacture of whey protein and milk protein concentrates and isolates.

Concentration

The two common delivery forms of dairy ingredients are liquid concentrates and powders. Starting with milk, fat separation generates cream, which is a concentrated milk fat source. The fat content of cream is controlled by the centrifugal operations and varies from 10% to 70%. In these concentrated milk fat sources, the serum content is between 30% and 90%. To minimize the serum content, cream is converted to butter by phase inversion. Butter has approximately 15% moisture. The residual moisture can be decreased in the manufacture of butter oil that has less than 1% moisture. The other product of milk separation is skim milk or milk serum. This product has more than 90% water and must be concentrated by partial removal of water. Products such as condensed skim milk, sweetened condensed skim milk, and skim milk concentrate are concentrated sources of milk-solids-not-fat (MSNF). These concentrated sources of MSNF still contain more than 55% water. Further reduction of moisture content is achieved by drying skim concentrate to a solid with less than 4% moisture, a product sold as non-fat dried milk.

Milk is concentrated by vacuum evaporation. Water removal by atmospheric boiling leads to denaturation of milk proteins and the development of an undesirable cooked flavor. Because the boiling point of liquids is lower when under pressures below atmospheric pressure, concentration can be performed at temperatures that do not produce deleterious effects. Designs for evaporators, which are used to concentrate milk, can be falling film, rising film, or plate; the former two use a shell-in-tube heat exchanger. When multiple

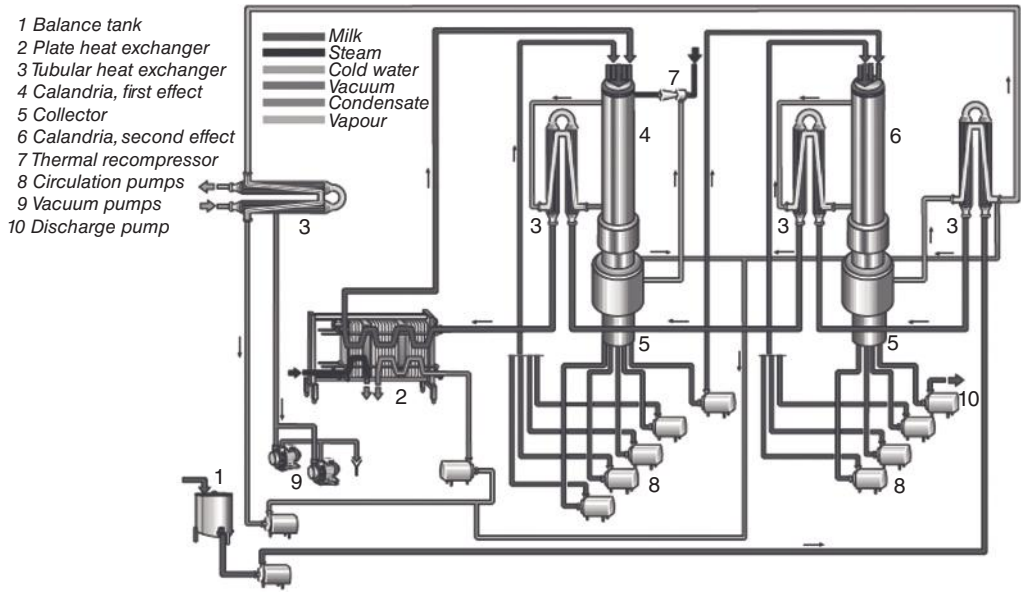

Figure 4.17. General schematic for a double effect evaporator with thermo-mechanical vapor recompression.

film evaporators are linked in a series they are referred to in terms of effects. For example, if three shell-in-tube evaporators are linked in series, they are referred to as a triple-effect evaporator. The temperature at which milk boils is a function of the extent of vacuum applied. In multiple-effect evaporators, each successive evaporator has a greater vacuum than the preceding unit. Vapor released from the concentration process is compressed and used as a heating medium. Such units are called evaporators with thermocompression.

Removal of water is an energy-intensive process, and thermocompression can minimize the energy used in the concentration process. A two-effect falling film evaporator with thermocompression requires about 0.25 kg steam to evaporate 1 kg of water. In a five-effect evaporator with thermocompression, 0.20 kg of steam is required for each kilogram of water evaporated. Without thermocompression, 0.6 and 0.4 kg steam, respectively, would be needed for the removal of one kilogram of water. Milk is concentrated to a maximum of 48% to 52% solids or an approximately four-fold concentration. General schematics for dual- and triple-effect evaporators are shown in Figure 4.17.

Three different types of concentrated dairy products are commonly used as ingredient: concentrated, evaporated, and sweetened condensed milk. The three types of non-fat concentrated forms are also available as nonfat concentrate, evaporated, and nonfat sweetened condensed milks. Details of these products are discussed elsewhere (Chapter 5).

Drying

Many dairy ingredients are sold as a dry powder with a moisture content of approximately 3%. If moisture content is above 5% the shelf life of dairy powders is drastically reduced; Maillard browning reaction occurs and caking and lumping is increased. Maillard browning not only affects color but also is responsible for off flavor development. Drying is the final step in ingredient manufacture. Removal of moisture is an expensive

proposition and if milk or skim milk were to be dried directly, the resulting powder would be very fine and the cost of drying prohibitive.

Concentration (see above) precedes the drying step, in which a four-fold concentration of solids is achieved by economical means. The concentrate is the feed material that introduced into the dryer. Moisture removal involves either roller or spray drying.

Drum or Roller Drying

In roller drying, milk or milk products can be dried in a thin film on an internally steam-heated drum and the dried product removed from the exterior of the drum by a doctor blade. Roller drying requires less space, can be installed in existing facilities, is made of equipment that is relatively simply constructed, is easy to commission, is easy to clean, and can handle sticky products (e.g., mixtures of cereals and milk). The steam consumption is 1.1 to 1.2 kg/kg of water evaporated. The major disadvantages of the roller dryers are: the solubility of the powders is less than that obtained from spray drying, they have greater amounts of scorched particles, and the solids concentrations of the feed are lower. Roller drying is not suitable if high-drying capacities or high-powder solubility are desired. However, there are some niche uses of this technology. Roller dryers often manufacture milk powder for use in meat applications in which high water binding capacity is desirable. High water binding is the result of the denaturation of milk proteins in roller drying. Roller-dried powders also are desired by chocolate manufacturers because of the high free fat content of these powders.

Roller dryers are classified according to the number of drums, method of placing the product on the surface of the drum, and the pressure surrounding the drum. In either type of drum dryer (single or double; atmospheric or vacuum) the feed (material being dried) can be applied in several ways. The feed can be sprayed on drums, placed on the bottom by an applicator roll or placed on the top of the drum by several applicators (Figure 4.18).

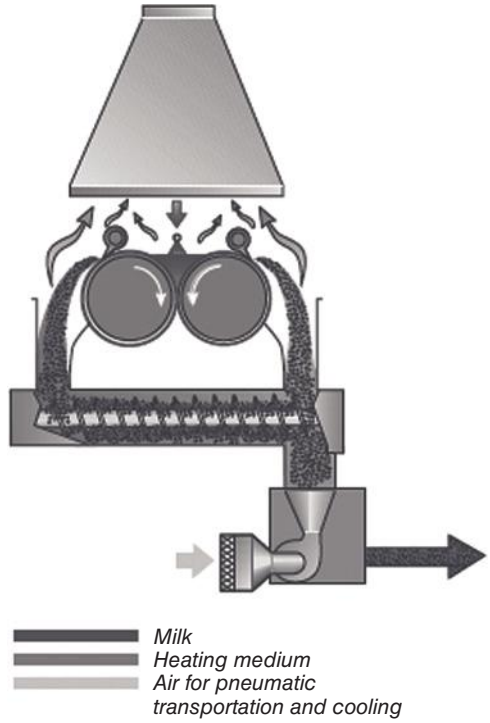

Figure 4.18. Schematic of a trough-fed roller dryer. Reproduced with permission from Tetra Pak.

Spray Drying

Spray drying is the transformation of liquid dairy products into powders by spraying liquid into a controlled flow of hot air within a drying chamber. It is a single-step continuous process. Spray-dried power consists of single particles or agglomerates and the final properties of the product depend upon the physical and chemical properties of the feed material as well as the dryer design and operation. The feed is pumped from a balance tank to an atomizing device in the drying chamber. The drying air is drawn from the atmosphere and filtered and heated. The

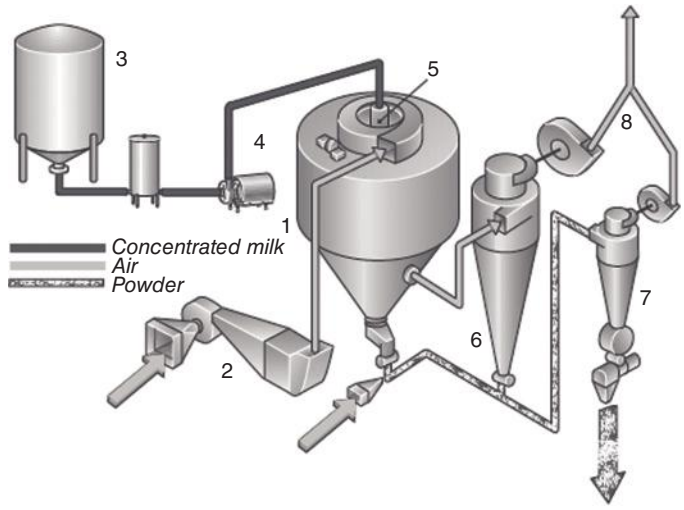

Figure 4.19. Schematic of a spray drying unit. Reproduced with permission from Tetra Pak.

spray of atomized droplets meets the hot air and spray evaporation takes place, simultaneously cooling the air. After the drying of the spray in the chamber, the dry product is separated from the exhaust drying air (see details in Chapter 6). Drying is also accomplished in two or three stages to allow for instantization (agglomeration) of particles. Agglomerated powders hydrate with ease. A general schematic is provided in Figure 4.19.

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Chapter 5

Concentrated Fluid Milk Ingredients

Nana Y. Farkye and Shakeel ur-Rehman

Introduction

Concentrated fluid milk products can broadly be defined as liquid or fluid milk that has had its composition altered from its normal, natural state by techniques such as evaporation, condensing, and membrane processing (e.g., ultrafiltration [UF], reverse osmosis [RO], nanofiltration [NF], microfiltration [MF], or combinations thereof). The resultant fluid milk product usually has higher total solids than normal milk but the concentrations of its components differ.

Concentrated milk offers economy, reduced transportation costs, convenience, added utility, and functionality (nutrient dense) to producers, processors, and consumers. For example, milk may be concentrated 3 : 1 fold on the farm prior to shipping to a milk processing factory, permitting a tanker to haul 3 times the original milk volume/weight for the same cost. The highest practical concentration for milk to be sold as fluid milk is 3 : 1 on a volume basis. At this concentration, the lactose content is approximately 13.1%, which is equivalent to 19.9 g lactose/100 g water, making lactose saturated at 21°C; it may be supersaturated at refrigerated temperatures. Hence, higher concentration levels may result in lactose crystallization. At a 3 : 1 concentration, 946.38 ml (1 quart) of concentrated milk testing 9.92% fat and 24.10% solids-not-fat (SNF) (34.02% total solids, TS), and weighing 2.275 lbs upon addition of approximately 1.9 L (2 quarts) of water yields 3 quarts of reconstituted milk testing 3.5% fat and 8.5% SNF.

Composition of Liquid or Fluid Milk and Market Milk

Milk is defined by the Code of Federal Regulations (21CFR 131 : 110) as a lacteal secretion from complete milking of one of more healthy cows, containing at least 3.25% fat and at least 8.25% SNF. Physical fractionation of milk resulting in removal of water or altering the relative concentrations of milk components to increase TS content results in a product that is broadly defined as "concentrated fluid milk" in this chapter. The reader must be aware of the legal definition of concentrated milk as defined in the Code of Federal Regulations (21CFR131.115).

Changes Caused by Concentrating Milk

In addition to the increase in solute concentrations that results from concentration of milk due to removal of water, other changes occur in milk. Walstra et al. (1999) list the following changes that result from concentrating milk:

1. Decrease in water activity (e.g., water activity of milk is 0.993 compared to 0.986 for evaporated milk).

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2. Increase in hygroscopicity.
3. Changes in salt equilibrium in milk (mainly due to increased Ca^{2+} ion activity leading to undissolved calcium phosphate. Consequently, pH of concentrated milk decreases by 0.3 to 0.5 unit.
4. Conformational changes in proteins due to increased protein association and compaction.
5. Changes in physicochemical properties (i.e., osmotic pressure; freezing point depression; boiling point elevation; increase in electrical conductivity, density, and refractive index; and decrease in heat conductivity).
6. Increased viscosity, which is temperature dependent.
7. Decrease in diffusion coefficient with moisture content.

Historical Perspective for Evaporated and Condensed Milks

In the late 1700s Nicolas Appert was the first to develop the process to evaporate and preserve milk in a sealed container in France to meet the required need for the French army to preserve food. Appert was awarded a patent on January 30, 1810, for developing a process for keeping milk for an extended period. His process included boiling milk in an open kettle to one-third of its original volume, sealing it in an airtight bottle, and reheating the sealed container and its contents in a hot water bath. The reason behind the preservation that resulted from Appert's method was later explained by the work of Louis Pasteur, who demonstrated the nature and behavior of microscopic organisms during heat processing.

Further development by was reported in an English patent awarded in 1813 to Edward Howard, who described a vacuum pan method for boiling milk vigorously at a low temperature (54.4°C or 130°F) and removing 50% of its water content. This led to the vacuum pan method for manufacturing evaporated milk in England in 1835. In 1856, Gail Borden received patents in the United States and England for preserving milk in a semifluid state after evaporation in a vacuum, leading to sweetened condensed milk in hermatically sealed cans. In 1857 an English patent was granted to Joseph House for preserving unsweetened condensed milk. However, the first known commercial evaporated milk was produced in 1885 in the United States by the Helvetica Condensing Milk Company in Highland, Illinois.

The application of homogenization in the production of evaporated milk was introduced in 1909 to stabilize the emulsion and reduce fat separation. The continuous system for sterilizing evaporated milk was developed in 1922, and in 1923, the U.S. Department of Agriculture promulgated an advisory standard for condensed milk, evaporated milk, and concentrated milk. For more on the historical developments and perspectives, see Parfitt (1956) and Bell (1962).

Types of Evaporated and Sweetened Condensed Milks

Unsweetened and sweetened condensed milk can be made from fresh milk or recombined milk (nonfat dry milk, fat, and water). When the source of fat is other than butterfat, the resultant milk is called filled milk. The U.S. Code of Federal Regulations (21CFR131 : 115) defines concentrated, evaporated, and sweetened condensed milk as follows:

"Concentrated milk, also called condensed milk, by definition is product obtained by partial removal of water from milk. It contains not less than 7.5% milk fat and not less than 25.5% total milk solids. It is pasteurized but not processed by heat to prevent spoilage and it may be homogenized. Vitamin addition is optional. If added, the quantity of Vitamin D in each fluid ounce is 25 IU."

Evaporated milk, also called unsweetened evaporated milk, is about 2 times concen-

Table 5.1. CODEX standards for different evaporated milks.

	Evaporated milk	Evaporated skimmed milk	Evaporated partly skimmed milk	Evaporated high-fat milk
% Minimum milk fat	7.5	1	More than 1 but less than 7.5	15
% Minimum milk solids*	25	20	20	11.5
% Minimum milk protein in milk-solids–not–fat*	34	34	34	34

*Milk solids and milk-solids-not-fat content include water of crystallization of lactose.
Farkye (2008)

trated whole milk. It is made by removing about 60% of the water in milk. It contains not less than 6.5% milk fat, not less than 16.5% milk-solids–not–fat (MSNF), and not less than 23% by weight of total milk solids. It contains added vitamin D (25 IU/fluid oz, where 1 fluid oz = 0.0296L). It is heat sterilized to prevent spoilage. Optional ingredients may include vitamin A (125 IU per fluid ounce), stabilizers, and emulsifiers (21CFR131 : 130). International standards (CODEX STAN 281) (CODEX Alimentarius, 2007) specify four types of evaporated milks, which are given in Table 5.1.

Condensed milk may be produced from milk and milk powders, cream and cream powders, and milk fat products. The protein content is typically adjusted with lactose or with retentate or permeate obtained from ultrafiltration of milk, part skim milk, or skim milk. Other permitted ingredients include potable water, sugar, and sodium chloride. In addition, firming agents such as the following may be used at levels permitted by good manufacturing practices: potassium or calcium chloride (used at a rate of 2 g/kg singly or 3 g/kg in combination, expressed as anhydrous substances); stabilizers (e.g., sodium, potassium, or calcium citrates, used at the rate of 2 g/kg singly or 3 g/kg in combination, expressed as anhydrous substances) or acidifiers (e.g., carbonates and phosphates of sodium, potassium or calcium); di-, tri-, and polyphosphates (used at the rate of 2 g/kg singly or 3 g/kg in combination, expressed as anhydrous substances); carageenan (150 mg/kg) as a thickener; and lecithin as an emulsifier.

Sweetened condensed milk (SCM), also called condensed milk, consists of concentrated milk solids made by removal of about 60% water from a mixture of milk (whole, nonfat, or homogenized) and safe and suitable nutritive carbohydrates such as sucrose. It contains not less than 8% milk fat and not less than 28% total milk solids. The product is pasteurized and may be homogenized (21CFR131 : 120). International standards for sweetened condensed milk require a minimum fat content of 8% and a minimum of 28% total milk solids. The minimum sugar content is not specified but is usually above 40% and should be sufficient to prevent spoilage. Addition of fruit juices or concentrates, coloring, and natural and artificial flavors is permitted.

CODEX STAN 282 allows the use of the following raw materials in SCM: milk and milk powders, cream and cream powders, and milk fat products. Other dairy products used as raw material for protein adjustment include milk retentate, permeate, and lactose (also used for seeding). The products are used in such a way as not to alter the whey protein:casein ratio in the milk being adjusted. Permitted ingredients are potable water, sugar, and sodium chloride. According to the CODEX standards, the finished food contains not less than 8% by weight of milk fat, and not less than 28% by weight of total milk solids, and protein not less than 34% of the solids-not-fat (SNF).

The following food additives are allowed when used at levels permitted by good manufacturing practices: firming agents (potassium or calcium chloride, used at a rate of 2 g/kg singly or 3 g/kg in combination); stabilizers (sodium, potassium, or calcium citrates, used at a rate of 2 g/kg singly or 3 g/kg in combination) or acidifiers (e.g., phosphates and carbonates of sodium, potassium, or calcium); di-, tri-, and polyphosphates (used at rate of 2 g/kg singly or 3 g/kg in combination); carageenan (150 mg/kg) as a thickener; and lecithin as an emulsifier. CODEX standards allow for four types of sweetened condensed milk (Table 5.2).

The average compositions of evaporated and sweetened condensed milk compared to whole milk are given in Table 5.3.

Technologies for Manufacture of Condensed and Evaporated Milks

Evaporation is used to concentrate milk before drying or transportation. If milk is to be transported long distances, it is concentrated to 30% to 38% TS to reduce transportation costs. Evaporation is one of the most energy-intensive processes in the dairy industry. Hence, modern evaporators are efficiently designed for low energy consumption and to provide minimal heat damage to milk components.

There are basically two types of evaporator designs—tubular and plate—that may be single effect or multiple effects of two or more units. Listed below are various commercial types of evaporators.

Table 5.2. CODEX standards for different sweetened condensed milks.

	Sweetened condensed milk	Sweetened condensed skim milk	Sweetened condensed partly skimmed milk	Sweetened condensed high-fat milk
% Fat	8	1	More than 1 to less than 8	16
% Milk-solids-not-fat	Not specified	Not specified	20	14
% Milk solids*	28	24	24	
% Milk protein in milk-solids–not–fat*	34	34	34	34

*Milk solids and milk-solids-not-fat content include water of crystallization of lactose.
Farkye (2008)

Table 5.3. Proximate composition of whole, evaporated, and sweetened condensed milks.

Component	Whole milk	Evaporated milk	Sweetened condensed milk
% Protein	3.2	7.0	8.2
% Moisture	88	74	26
% Fat	3.5	7.5	8.0
% Carbohydrates	4.6	9.6	55.1
% Lactose	4.6	9.8	10–12
% Sucrose			44–46
% Ash	0.7	1.5	1.8
Calcium (mg)	120	228	238
Phosphorus (mg)	90	213	236
Sodium (mg)	53	94	88
Potassium (mg)	136	297	360

Farkye (2008)

- Falling film evaporators
- Rising film evaporators
- Forced circulation evaporators
- Plate evaporators
- Vapor recompression evaporators

Falling Film Evaporators

The falling film tubular evaporator is primarily used in the dairy industry. In this type of evaporator, liquid and vapors flow downward in parallel flow. The liquid to be concentrated is preheated to boiling temperature. An even and thin film of the liquid enters the heating tubes via a distribution device in the head of the evaporator, flows downward at boiling temperature, and is partially evaporated. This gravity-induced downward movement is increasingly augmented by the co-current vapor flow.

Falling film evaporators can be operated with very low temperature differences between the heating media and the boiling liquid, and they also have very short product contact times, typically just a few seconds/pass. These characteristics make the falling film evaporator particularly suitable for heat-sensitive products such as milk.

Rising Film Evaporators

In rising film evaporators, the feed enters the bottom of the heating tubes and as it gets heated, steam begins to form. The ascending force of the steam produced during the boiling causes liquid and vapors to flow upward in parallel flow. At the same time the production of vapor increases and the product is pressed as a thin film on the walls of the tubes, and the liquid rises. The co-current upward movement helps to create a high degree of turbulence in the liquid, making the process advantageous for evaporation of highly viscous products and those that have a tendency to foul the heating surfaces.

Falling film evaporators are often used with product recirculation, in which some of the formed concentrate is reintroduced back to the feed inlet to produce sufficient liquid inside the boiling tubes.

Forced Circulation Evaporators

Forced circulation evaporators are used if boiling of the product on the heating surfaces is to be avoided due to the fouling characteristics of the product, or to avoid crystallization. Forced circulation evaporators are usually for viscous liquids, corrosive liquids, and concentrated liquids that cause fouling and scaling problems.

Plate Evaporators

Framed plates can be used instead of tube bundles as a heating surface. These plate assemblies are similar to plate heat exchangers, but they are equipped with large passages for the vapor flow. In these units a product flow plate and a steam flow plate are connected alternately. The product passage is designed for even distribution of liquid on the plate surfaces and low pressure drop in the vapor phase.

Vapor Recompression Evaporators

To minimize energy consumption, two systems for vapor recompression are used: Thermal vapor recompression (TVR) and mechanical vapor recompression (MVR) evaporators. MVR evaporators are single-effect evaporators divided into several stages (approximately five to eight), using a high-pressure fan for recompression of vapor. Thus, the heating medium in the first effect of MVR evaporators is vapor developed in the same effect, compressed to a higher temperature using turbocompressor or high-pressure fans. TVR evaporators are multiple-effect evaporators with two to seven effects using a steam jet compressor for re-compression of

the vapor over one to three effects. In TVR evaporators, the heating medium in the first effect is the product vapor from one of the next calandria, compressed to a higher temperature by steam injection. Vapor generated from each calandria is the heating medium in the next, while vapor from the last effect is condensed and may be used as boiler or cleaning water or to preheat incoming air for spray drying. For further reading, see Hess (1993), Carić (1994), Walstra et al. (1999), and Niro Inc. (2007).

Process for Manufacture of Evaporated Milk

The manufacture of evaporated milk involves the following steps:

1. Preparation (i.e., standardization, heat treatment, concentration/evaporation, homogenization, and cooling) of a concentrated milk
2. Canning and heat sterilization of the concentrate
3. Cooling and storage

For good-quality evaporated milk, incoming raw milk must be of good microbial and organoleptic quality. Although the regulatory limits for Grade A raw milk in the United States are microbial counts less than 100,000/ml, coliform counts not exceeding 10/ml, and somatic cell counts of less than 750,000/ml, most Grade A raw milk produced on dairy farms in many regions of the United States may be less than 10,000 colony-forming units (cfu)/ml.

The raw milk is clarified and filtered to remove any debris or foreign matter and standardized to the desired fat content. For a stable product and to prevent coagulation during heat processing, and to minimize age thickening during storage, the milk is heat stabilized by adding small amounts of stabilizing agents such as phosphates, citrates, and bicarbonates to maintain pH 6.6 during 6.7 during heat treatment. More commonly, both disodium orthophosphate (DSP) and monosodium orthophosphate (MSP) are used. DSP and MSP have opposite effects on the pH of the milk. Hence, the appropriate phosphate is used to give the evaporated milk the pH of optimum heat stability. The phosphate salts also may limit binding of ionic calcium to casein micelles and reduce the possibilities for subsequent precipitation (Fotheringham and Choat, 1979). Carrageenan is also added as a thickener.

The heat stability of milk is influenced by several compositional factors including mineral (ash) content and protein and acidity levels. Natural heat stability also varies seasonally and is influenced by stage of lactation.

Preheating

Preheating reduces the microbial load, inactivates some indigenous milk enzymes, and enhances heat stability (i.e., increasing the resistance of the milk to coagulation during subsequent sterilization.) In addition to the primary purpose of increasing heat stability, preheating also affects viscosity of the final product. Preheating is done in continuous heat exchangers (plate or tubular) with long holding times. The time and temperature requirements are usually 93°C to 100°C (199°F to 212°F) for 10 to 25 minutes or 115°C to 128°C (239°F to 262°F) for 1 to 6 minutes.

Concentration

Next, the heated milk is evaporated under vacuum (typically using any of the multiple-effect evaporator types described earlier). Evaporation under vacuum exposes milk to a pressure lower than atmospheric pressure and its boiling point is lowered to approximately 45°C (113°F). Typical evaporation temperatures used are not less than 45°C (seldom

exceeding 54.5°C; 130°F) to prevent growth of microorganisms. Evaporation under partial vacuum also helps prevent undesirable changes to milk components (e.g., heat damage to milk proteins). The milk is concentrated to 30% to 40% TS. Although concentration by evaporation is the most common method, the milk also may be concentrated by reverse osmosis, a membrane filtration process used in the dairy industry to remove water from milk and whey at lower energy costs compared to evaporation.

Homogenization

Next, the milk is homogenized at high pressure, i.e., 15 to 25 MPa (200 to 250 bar) first stage and 5 to 10 MPa second stage. The preferred homogenization temperature matches the evaporation temperature of more than 45°C (113°F). Homogenization prevents creaming and coalescence. It breaks down the fat globules from an average size of 3 to 5 µm or larger into smaller sizes of less than 1 µm, resulting in improved color (natural white to light cream color) and stability of the milk. However, excessive homogenization pressure may result in an irreversible destabilization effect and reduction in heat stability of the product. Following homogenization the product is cooled and placed into storage, where the final standardization of composition takes place.

Second Standardization and Stabilization

During the second standardization and stabilization, the total solids content in the product is readjusted to meet required standards of the finished product. The process of standardization can take several hours because the evaporated milk in storage tanks must be analyzed prior to standardization. Water, skim milk, evaporated milk, retentate, permeate, and homogenized cream are often used to standardize of the fat:SNF ratio. The salt balance in the milk, hence pH, also is adjusted with stabilizing salts to ensure that the product withstands further intense heat treatment. Because the salts are added in aqueous form, the milk is concentrated to higher TS content so that re-standardization and stabilization is used to bring the solids content to desired levels.

Packaging

The most common method for packaging evaporated milk is canning with a suitable filling machine with lid seaming. Typically the can is manufactured with a lid that has a center hole (diameter 2 to 3 mm), through which the can is filled. Necessary precautions are taken during filling to prevent foaming. After filling, the can is immediately sealed hermetically (i.e., avoiding the passage of air or fluid in either direction) by soldering. Because the milk is cold before it is filled in the can, sufficient head space equal to approximately 10% of the can volume must be allowed for product expansion during sterilization. A large head space may result in excessive foaming, clotting, and brown deposits forming around the corners of the can. Hence, the head space can be partially evacuated by the injection of steam into the head space of the can at the time of closing. A suitable vacuum level is 2 to 4 inches of Hg (6.773×10^3 to 1.355×10^4 Pa) in the can after cooling.

Sterilization

The canned product is sterilized *in situ* at 115°C to 121°C (239°F to 250°F) for 15 to 20 minutes followed by cooling for about 15 minutes to 25°C to 30°C (77°F to 86°F). This is called in-container sterilization. It can be done in batches or continuously. Sterilization kills bacterial spores and inactivates heat-stable indigenous milk enzymes such as

plasmin and other bacterial enzymes that may be present in the milk. The adequacy of sterilization is checked by holding random samples of the cooled canned evaporated milk for two to three weeks at 27°C to 30°C (80°F to 86°F) and checking for spoilage (i.e., microbial growth, gas formation, bulging and explosion of cans) before shipping. When product is to be shipped to warmer climates, incubation of the cans may be done at 37°C (96.8°F) or 55°C (131°F) to detect facultative or obligate thermophilic bacteria, respectively.

Evaporated milk must be commercially sterilized to have an acceptable shelf life at room temperature, which means that it must not contain organisms that will grow under normal storage conditions. Although obligatory thermophilic organisms may grow at high temperatures such as 45°C (113°F), the product is still considered commercially sterile. A time-temperature combination to give absolute sterility is possible; however, the resultant product has an unacceptable cooked flavor and a dark color resulting from Maillard browning.

Sterilization may also be done by ultra-high-temperature (UHT) heating at 130°C to 140°C (266°F to 284°F) using direct or indirect heating. After sterilization, the cans are sealed aseptically. Aseptic packaging occurs under pressure at temperatures exceeding 100°C.

Spoilage of Evaporated Milk

Sterilization is designed to kill heat-resistant spores, most of which in milk are species of the genus *Bacillus* or occasionally *Clostridium*. However, inadequate cooling and/or storage at high temperatures may cause growth of some heat-resistant spores. The most heat-resistant spore in milk is *Bacillus stearothermophilus*. This organism may not grow in temperate climates but grows well under tropical conditions. *Bacillus stearothermophilus* grows best at 37°C (98.6°F) and above, and may cause acid coagulation and a slight cheesy odor. *B. subtilis* causes non-acid curd with a bitter taste. *Clostridium sp*. causes the putrefaction and gas production with a odor of H_2S.

Manufacture of Sweetened Condensed Milk

Sweetened condensed milk is manufactured from whole milk, skim milk or recombined condensed milk (consisting of skim milk powder, anhydrous milk fat or vegetable fat, and water). The processing steps in the manufacture of SCM are as follows:

1. Standardization of milk
2. Heat treatment of milk
3. Evaporation
4. Addition of sugar
5. Cooling
6. Seeding and subsequent cooling for crystallization
7. Canning and packaging

Raw milk of good microbiological quality and low spore counts is the preferred starting material. When nonfat dry milk (or skim milk powder) is used for recombined SCM, it must also have a good microbial quality and low spore counts.

Standardization

The raw milk is standardized to 8% fat and 21% SNF, giving a fat:SNF ratio of 0.381. Standardization is achieved using any of the ingredients described in the CODEX standard or in the respective country. Following standardization, the milk is given an initial heat treatment (80°C to 120°C; 176°F to 284°F) similar to that used for evaporated milk manufacture. The heat treatment influences viscosity of the final product and age thickening during storage. Heat treatment in the range of 90°C to 100°C (194°F to 212°F) gives the product its greatest susceptibility to

age thickening during storage. In general, low heat treatments favor increased viscosity, whereas high heat treatments give lower viscosity in SCM. Heating at 80°C to 85°C (176°F to 185°F) for 15 to 25 minutes gives the desired initial viscosity with slow age thickening. Viscosity should be sufficient to prevent separation during storage and age thickening; the product should remain pourable during storage. The average viscosity of SCM is approximately 2 Pa.s, about 1,000 times the viscosity of milk. The heated milk is homogenized at low pressure (approximately 2 to 6 MPa) because creaming is not often a problem in SCM.

The heated product is then condensed by evaporation under vacuum at 65°C to 70°C (149°F to 158°F) in a multiple-effect evaporator. Reverse osmosis may also be used to concentrate the milk. Condensation temperatures lower than 65°C (149°F) favor germination of spores and growth of heat-resistant bacteria. The concentrated product is then cooled to 20°C to 30°C (68°F to 86°F) using vacuum coolers.

Because SCM is not heat sterilized, the addition of sugar serves improves its keeping quality by providing a bacteriostatic environment. Sucrose is the preferred sugar used in SCM manufacture, although glucose and other sugars have been used for diabetic products. The sucrose is added in crystalline form or as a solution by dissolving it in water at about 95°C (203°F) before adding it to milk by high shear recirculation in the milk. The time of sugar addition influences product quality. Adding sucrose before heat treatment increases heat resistance of bacteria and their enzymes, leading to age thickening, and adding sucrose before evaporation causes increased viscosity. Hence, the best time for adding sucrose to give optimal product quality is near the end of evaporation. The final concentration of sugar in the aqueous phase of SCM, known as the sugar number or sugar index, is approximately 62.5% to 64.5%.

After addition of sugar, the fat and total solids content of the product is re-adjusted to desired levels to meet minimum standards.

Lactose Seeding

Next, lactose crystallization is induced. Although the concentration of sugar in moisture is above 61%, osmophilic organisms can grow. Because part of the lactose contained in the product may be over saturated, autocrystallization of lactose may occur and lactose may appear as large crystals with a size greater than 15 μm. The presence of large crystals results in a defect known as sandiness, i.e., a gritty mouth feel when SCM is consumed. To avoid sandiness, the concentrate is cooled to the optimal seeding temperature of approximately 25°C to 30°C (77°F to 30°F) and inoculated or seeded with fine milled and pasteurized dry lactose crystals to promote instant and controlled crystallization. Below 20°C (68F°F), lactose crystallizes instantly without seeding. At 30°C to 50°C (86°F to 122°F), less lactose crystallizes and above 50°C (122°F), lactose is in solution and does not crystallize. The amount of lactose added is equivalent to 0.5 kg/1,000 kg milk. The smallest possible size for the seed lactose should be less than 10 μm with a significant portion less than 1 μm. Rapid cooling and agitation occurs during seeding. The amount of crystals formed is more than 4×10^{11} crystals/m^3. Lactose crystal size affects viscosity of SCM. During the first half of crystallization, the viscosity of SCM increases to a maximum because of small crystal size but as crystal size grows, viscosity decreases.

After crystallization is complete, the product is packaged by filling tin cans that have been previously sterilized by flaming. It is important that the air in the filling area is clean and filtered to avoid future microbial quality issues. Furthermore, the filled can must have the lowest possible air space (head space) above the product to prevent mold growth.

Dulce de Leche

Dulce de Leche (caramel jam) is an Argentinean dairy product that is popular throughout Latin America. It is a form of sweetened condensed milk prepared by boiling whole milk with added sucrose until 70% (wt/wt) total solids is reached (Monro and Hough, 1985). The typical starting formulation is 10 parts milk and 2 parts sucrose. Sucrose usually is replaced partially by glucose syrup to avoid crystallization. About 0.1% sodium bicarbonate is added to increase browning and prevent protein coagulation. The lactose concentration in dulce de leche is 9.85% from milk with 12% total solids and 4.5% lactose (Hough et al., 1990).

Defects in Sweetened Condensed Milk

Microbial Spoilage

Because SCM is not a sterile product, it is prone to microbial spoilage, although its low water activity (approximately 0.83) and high sugar content prevent microbial growth. However, osmophilic yeasts of genus *Torulopsis* may grow and cause gas formation and bulging cans. They also cause coagulation and produce fruity flavors. Other microorganisms that grow in SCM are micrococci and molds such as *Aspergillus repens* and *Aspergillus glaucus,* and *Penicillium* sp., which grow on the surface of SCM when sufficient air (and oxygen) is available.

Chemical Defects

Age thickening is the main chemical defect in SCM, followed by gelation. Factors that affect chemical defects include seasonal variation in milk composition, preheat treatment of milk, the stage at which sugar is added, the degree of concentration, and stabilizing salts added. For example, early lactation milk is more sensitive to age gelation than mid-lactation milk; less age thickening occurs when milk is heated by UHT treatment with long heating times; late addition of sugar during the evaporation step reduces age thickening; and a high concentration factor increases age thickening. Age thickening also is influenced by the type, amount, and stage at which stabilizing salts are added. For example, adding 0.03% sodium tetra pyrophosphate delays age thickening, whereas adding more promotes age thickening. Age thickening increases with storage temperature (Q_{10} = approximately 3.4) and in tropical climates, SCM gelation occurs in about one year. High temperature storage may also lead to brown discoloration due to Maillard reaction.

Recombined Evaporated and Sweetened Condensed Milk

In countries with a limited milk supply, evaporated milk and SCM are manufactured using nonfat dry milk (NFDM) or skim milk powder as the starting material. Other ingredients include sweet cream buttermilk powder, fat (anhydrous milk fat [AMF] or vegetable fat), water, and stabilizing salts. When evaporated milk or SCM are manufactured using all dairy ingredients, the products are called recombined evaporated or recombined sweetened condensed milk, respectively. When vegetable fat is used instead of milk fat, the products are described as filled milk (Fotheringham, 1979). The specific requirement of the NFDM used for manufacture of evaporated and sweetened condensed milk is that it must be heat stable (i.e., must not coagulate when subjected to intense heating). There are three different classes of NFDM based on their relative concentrations of undenatured whey protein nitrogen, and these are expressed as the whey protein nitrogen index (WPNI). The American Dairy Products Institute (ADPI) (ADPI, 2002) classification of NFDM is given in Table 5.4.

The most preferred NFDM used for sterilized milk products are medium- or high-heat

Table 5.4. Heat classification of nonfat dry milk.

Class	Whey protein nitrogen (mg/g)
Low heat	Not less than 6
Medium heat	1.51–5.99
High heat	Not more than 1.5

ADPI (2002)

NFDM that are manufactured from skim milk that has been respectively preheated to 82°C for 3 minutes or 82°C for 30 minutes before evaporation and drying. Low-heat NFDM is more suitable for nonsterilized products such as sweetened condensed milk. After reconstitution of the dried milk in water and recombination with milk fat or vegetable fat by high shear mixing and homogenization, the processing steps similar to those described above for evaporated milk and sweetened condensed milk are followed.

Quality Assessment

The quality of concentrated milks depends on the quality of the starting raw milk, other ingredients added, and processing conditions including adherence to strict sanitation and good manufacturing practices. In the United States, raw milk quality must follow Grade A standards specified by the PMO (FDA, 2003) as follows:

Temperature: Cooled to 10°C (50°F) or less within four (4) hours or less, of the commencement of the first milking, and to 7°C (45°F) or less within two (2) hours after the completion of milking. Provided, that the blend temperature after the first milking and subsequent milkings does not exceed 10°C (50°F).

Bacterial limits: Individual producer milk not to exceed 100,000 cfu/mL prior to commingling with other producer milk. Not to exceed 300,000 cfu/mL as commingled milk prior to pasteurization.

Drugs: No positive results on drug residue detection methods

Somatic cell count: Individual producer milk not to exceed 750,000 per mL.

SCM must meet strict microbiological standards as follows:

- Standard plate count (SPC): less than 1,000/g
- Coliform: less than 10/g
- Yeast: less than 5/g
- Mold: less than 5/g

The recommended microbiological testing methods for SPC, coliform, yeast and molds, and thermoduric and thermophilic counts are described in the Standard Methods for the Examination of Dairy Products (APHA, 1992).

Other Tests

There are several methods for assessing the quality of evaporated milks and other concentrated milks. Some of the more frequently used methods are described below. For a detailed description of the methods as well as other methods, see *Methods for Quality Assessment of UHT Milks* (New Zealand Dairy Research Institute, 1997).

Gelation

Gelation may be detected by visual inspection and is often rare within 6 months of production. Gelation may be indicated by wheying-off and shrinkage of the gel away from the wall of the container, failure of the surface to flow when the container is tipped, or the presence of curdy lumps when the sample is disturbed.

Fat Separation

Fat separation is evidenced by a cream layer on top of the product. Fat separation can be assessed by visual subjective, gravimetric (weight test), or fat emulsification methods. When the visual method is used, a

ream layer may not be noticeable in well-homogenized milk before the products is 2 months of age.

Sedimentation

Sedimentation is a subjective visual test. It is the sediment observed at the bottom of a package after pouring out the product compared to an internal standard chart developed with different degrees of sediments. Alternately, sedimentation is calculated as the weight difference of the container before and after rinsing off sediment.

Viscosity

The viscosity of concentrated milk is higher than that of normal milk and is in the range 15 to 60 mPa.s (cP). It is affected by fat and protein contents of milk and processing conditions such as heat treatment. Typically the test is done using a Brookfield viscometer equipped with spindle number 2 at 60 rpm. The temperature of the concentrate is kept at 40°C (105°F) ± 0.5°C.

Coffee Sediment

In the coffee sediment test, the addition of evaporated milk to hot coffee is simulated and the quantity of sediment formed after centrifugation is measured. The results of the test are influenced by the composition of coffee, pH, temperature, and quantity of water used to make the coffee. Therefore, it is important to standardize the method, including the type and brand of coffee used, to obtain reliable results.

Coffee Whitening

The coffee whitening test is similar to the coffee sediment test but instead of centrifuging out the sediment, the color is measured using a reflectance colorimeter. In addition to the precautions listed for the sediment test above, it is important that the coffee be freshly made because the color of coffee darkens on standing.

Membrane Concentration Technologies

The application of membranes to concentrate milk has become increasingly important in the dairy industry. Unlike evaporation, it is a technique used to concentrate milk with little or no heat treatment. The milk components are separated based on their molecular mass and shape under pressure using specially designed semi-permeable membranes. The different membrane processes used in the dairy industry are microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). During concentration by membrane processes, the portion of the liquid that passes through the membrane is called permeate, and the concentrated liquid retained by the membrane is called retentate.

The word membrane, derived from Latin, means skin. Membrane technologies have been used in the dairy industry for more than 30 years (Grandison and Clover, 1997). UF and RO systems were first reported to be used in the dairy industry in 1975 (Bargeman et al., 2005). NF was commercially introduced in the dairy industry around 1984 to simultaneously concentrate and demineralize whey; it is a process between UF and RO.

MF is a low-pressure membrane separation process using membranes of fairly large pore size and small pressure differential, thereby allowing particles greater than 0.1 μm (0.05 to 10 μm) to pass through. Thus, it is used to remove small suspended particles and microorganisms from milk.

UF is a selective fractionation/concentration process using pressures up to 145 psi (10 bar) to separate macromolecules and suspended solids with molecular weight

Table 5.5. Mean composition of protein, fat, ash, and lactose in UF skim milk at various concentration factors.

	Concentration factor of UF skim milk					
	1.5×	2×	2.5×	3×	3.5×	4×
% Protein	4.48	5.97	7.47	8.96	10.45	11.94
% Fat	5.51	7.34	9.18	11.01	18.85	14.68
% Ash	0.95	1.18	1.40	1.63	1.86	2.09
% Lactose	4.59	4.41	4.23	4.04	3.86	3.68

Table 5.6. Composition of different skim milk concentrates of membrane technology.

	Skim milk (1×)	Skim milk–RO concentrate (3×)	Skim milk – UF concentrate (3×)	Skim milk–MF concentrate (4×)
% Protein	3.2–3.3	9.6–9.9	9.6–9.9	12.0–13.0
% Lactose	4.8–5.0	14.4–15.0	4.8–4.9	1.1–1.2
% Fat	0.08–0.10	0.24–0.30	0.24–0.30	0.3–0.4
% Minerals	0.70–0.72	2.10–2.16	1.2–1.3	1.8–2.0
% Solids-not-fat	8.70–9.0	26.0–27.0	15.6–16.6	15.0–16.0
% Total solids	8.78–9.1	26.3–27.3	15.80–16.80	15.3–16.4

× = Concentration factor.

greater than 1,000 Daltons. It is effectively used to concentrate proteins (casein micelles), fat, and bacteria from milk. The concentrated product has a composition markedly different from evaporated skim milk and therefore different properties (Table 5.5). The UF milk has reduced Maillard browning during heating due to reduced lactose content; it is more heat stable at identical protein content and has higher viscosity at identical TS content than milk concentrated by evaporation.

Membrane processes are based on cross flow or tangential flow filtration technologies for continuous flows of liquid streams such as milk. MF, UF, and NF concentrate and fractionate milk components, whereas RO is a concentration process (Table 5.6). MF and UF processes concentrate on the basis of a sieving process; the molecules that are too large to pass though the pore are rejected and exist in the concentrate stream while the smaller molecules that pass the pore size of the membranes end in permeate stream. The largest and smallest molecules in milk are fat globules (2,000 to 10,000 nm) and water (0.3 nm), respectively. Fat globules are retained by all types of membranes currently used in milk processing, whereas water is retained by none. Casein micelles with a molecular size of 25 to 130 nm are retained by the membranes of MF, UF, NF, and RO.

Membrane processing of milk results in a concentrated or fractionated milk stream that has been obtained with little or no heating. Processing milk by membrane technology is ecologically friendly and gives high-quality products with improved taste and shelf life compared to the original milk. Use of membrane technology allows modifications of milk in such a way that the protein:lactose or protein : fat ratio can be adjusted very easily in the concentrated milks.

MF is used to separate and concentrate large molecular size casein proteins of skim milk. The size of MF membranes ranges from 0.1 to 1 microns. Two streams of liquid milk beverages are obtained as a result of MF, one rich in casein proteins and another rich in whey proteins. Skim milk can be fractionated by MF into a casein-rich concentrate

and serum-protein-rich permeate. The casein-rich concentrate can be used in fortifying cheese milk during cheese making or for making milk beverages or micellar casein. The serum-protein-rich permeate stream can be concentrated by RO to make milk beverages that do not contain caseins. The whey protein beverages resulting from MF and RO processing can have protein contents similar to those found in milk. Such beverages are suitable for individuals who are allergic to caseins. A combination of MF and UF would be needed if reduced-lactose whey-protein fresh milk beverages are to made.

UF is a membrane process in which proteins and fat in whole milk are concentrated. UF membranes in the dairy industry are rated by a molecular weight cut off, the maximum molecular weight of the substance that will pass into the permeate stream. UF membranes are usually smaller than 0.1 microns. MF and especially UF membranes retain all the fat and practically all the protein in milk. The retention coefficients of the non-protein nitrogen compounds are generally 20% to 40%, and higher for the high concentration factors (Grandison and Glover, 1997). Urea and amino acids are mainly lost through the membrane. Retention of lactose during UF may be up to 10%. The minerals and other ions retained during the membrane processing of milk by UF are those that are attached to the proteins, such as calcium, magnesium, phosphate, and citrate, whereas others pass into the permeate. Likewise, the fat-soluble and protein-bound vitamins are retained completely.

UF processes are used commercially to modify the proportion of lactose in milk and milk products. The concentrated milk products produced due to UF or MF have resulted in lactose-modified ice creams, milk powders, yogurts, and a series of fluid milks and dairy beverages. The addition of UF concentrate of milk changes the physical and chemical properties of all the dairy products to which it is added. During UF processing of milk to 3× concentration, 66% to 67% of lactose passes into permeate, and 33% to 34% is retained in the UF milk concentrate.

UF milk concentrates have been used to increase the protein content of fluid milks (Quinones et al., 1997), and it has been found that an increase of 0.9% in true protein content of fluid milk resulted in detection of perceivable sensory attributes. Addition of fresh UF milk concentrate to fluid milks for increasing protein content improves the flavor and mouth feel of fluid milks naturally, compared to adding NFDM. Addition of NFDM or condensed milk (CM) to fluid milks for increasing protein content results in a cooked flavor and increases sweetness from excess lactose in NFDM or CM. NFDM also requires additional equipment for mixing with fluid milks. Addition of UF milk concentrate to ice cream mix may reduce sandiness (caused by lactose crystallization) during ice cream storage.

Diafiltration (DF) is used to remove lactose from UF milk. During DF, water is added to the milk or to the UF milk to wash out lactose, minerals, and other small molecules that can pass through the membranes. The combination of UF and DF is used to produce concentrated milk that is high in protein and fat, and low in lactose and salts. The use of UF concentrates in the manufacture of SCM eliminates the sandiness defect (Sepraalverez et al., 1979) because of the low lactose content of the condensed milk.

NF is often considered loose RO. NF membranes concentrate multivalent salts to a higher degree than monovalent salts in milk. The molecular weight cut off of NF membranes is less than 1,000 Daltons. The main application of NF in the dairy industry is for concentration and demineralization of whey along with other filtration processes. However, NF has been used for skim milk modification (Nguyen, 1996). Hinrichs (2000) compared the heat stability of milk concentrates made with UF, RO, and NF, and reported that NF concentrates were intermediate in the heat stability, between RO and

UF. NF also has been commercially used to produce lactose concentrate from permeate resulting from UF of skim milk. Use of NF in such applications removes most of the monovalent minerals from the UF permeate of milk, resulting in total solids of 20% to 22% in the NF retentate.

The lactose concentrate may be processed into animal feed. It may further be purified into pharmaceutical grade lactose or hydrolyzed into glucose and galactose using β-galactosidase (lactase) and used in confectionery. Lactose is one-third as sweet as sucrose; therefore, three times more lactose must be used to give identical sweetness of sucrose, making it impractical to use lactose as a sweetener in foods. However, lactose may be used as a bulking agent. In baking and confectionery industries, lactose is used to produce a brown color and caramelized flavor. Because lactose is a reducing sugar, it reacts with ε-amino acids of proteins to cause browning to Maillard reaction, resulting in a caramel flavor.

RO is a concentration process in which 99% to 100% of the milk solids are concentrated in the retentate, whereas the RO permeate is essentially water. RO membranes have a molecular weight cut off of 50 to 100 Daltons. Use of RO to increase the concentration of solids of whole milk is finding increased acceptance in the dairy industry because the RO process does not change the flavor of whole milk like traditional evaporation process does. The major attraction for the dairy industry to energy savings. In the RO process, water is removed without change of phase or having to use extremes of temperature as in traditional evaporation or freeze concentration. Furthermore, the milk is exposed to minimum heat during concentration, which avoids protein denaturation, development of a cooked flavor, and the heat-damaging effects on the constituents of milk. The RO concentrates can be reconstituted back into fresh fluid milks by adding water from a dispenser (Figure 5.1).

Figure 5.1. A dispensing machine for reconstituting RO concentrate into fluid milk.

UHT Processed Milk Concentrates

Muir (1984) conducted a detailed study on the effect of concentration on the rheology and heat stability of UHT-sterilized milk concentrates. It is well known that the higher the volume concentration ratio of milk by membrane technology, the denser the packing of fat globules and proteins. However, depending on the concentration process (RO, NF, UF) used, the lactose and salts may be concentrated (Hinrichs, 2000). The viscosity of milk increases with concentration, whereas its heat stability decreases. A concentration limit of 31% TS for making UHT concentrates from whole milk was suggested by Muir (1992) because at concentrations higher than 31% TS, the heat stability is dramatically reduced and gelling is dramatically accelerated. Gelling of UF milk concentrates

of milk stored at 5°C (41°F)has been reported by de Carvalho (1986). Heat stability is better in UF concentrates than in evaporated milk concentrates (Sweetsur and Muir, 1980).

Hinrichs (2000) studied UHT concentrates manufactured by UF, RO, and NF. The UF and RO concentrates were produced from milk containing different levels of fat and protein. The heat stability at 140°C (284°F) and storage stability negatively correlated with the ash content. Hinrichs (2000) suggested that milk may be processed by UF and NF technologies to reduce its ash content, giving a high-quality milk concentrate that can heated by UHT to give a long shelf life. Lactose content did not influence the storage stability. The low lactose content of UF concentrates reduced the tendency of browning due to Maillard reaction during storage but affected the taste.

New Concentrated Fluid Milk as a Result of Using Combinations of Membrane Technologies

Select Milk Producers in the United States has patented a process for combining membrane technologies to manufacture lactose-free milks (Dunker et al., 2007; Shakeel-Ur-Rehman et al., 2007) sold under the trade name Mootopia™ in the United States. A concentrated milk product produced by using membrane technology and sweetened by honey, branded as Athletes Honey Milk, is becoming very popular in the U.S. market. This product is shelf-stable and contains 2.5 times the protein and minerals found in ordinary milk. These milks are considered to be concentrated milks because the protein content is greater than that found in fluid milk. The popularity of foods with a low glycemic value has also necessitated the manufacture of milk products with reduced milk sugar, but any reduction in lactose must be compensated for by increasing the protein content. Milk products in which the level of lactose is reduced using membrane technologies and compensated for by increased protein content may be suitable for diabetic people.

Other Uses of Concentrated Dairy Products

Concentrated milks have multi-functional purposes. They can be diluted for everyday milk use, e.g., cream for coffee or tea, poured on cereals and fruits, consistency in meat patties and loaves, coatings for baked or fried meats, or in place of milk in the manufacture of candies, frostings, and pies.

Concentrated fluid milk products are used by the food industry in many different types of food products because of their excellent nutritional and functional properties. Examples of such products are dietary foods, confectionery, baked goods, beverages, frozen desserts, cheeses, and other dairy products. Concentrated milk products can be used in whipped products in which foaming and frothing is desired. In cheeses, condensed skim milk or concentrates from membrane filtration are used to standardize cheese milk to boost yields. The principal functionalities on which their uses are based in various foods are given in Table 5.7.

Table 5.7. Examples of multiple functionalities required of various food products.

Food type	Multiple functionalities required
Beverages	Solubility, heat stability, pH stability, color
Baked goods	Emulsification, foaming, gelation
Meat emulsions	Emulsification, foaming, gelation
Soups and sauces	Viscosity, emulsification, water binding
Cheeses	Coagulability, yield enhancement
Confectionery	Viscosity, gelation, water binding
Frozen desserts	Foaming, gelation, emulsification
Sweetened condensed milk; dulce de leche	Viscosity, color, water binding

Applications of Concentrated Milks in the Baking Industry

In the baking industry, UF and RO concentrates of skim or whole milk are becoming popular as milk solid sources compared to skim milk powders or milk protein concentrate powders, due to their ease of mixing. Milk powders and milk protein concentrate powders are difficult to reconstitute and may need additional equipment for mixing; therefore, the concentrated milks are the ingredients of choice for the baking industry. The use of UF and RO milk concentrates in the baking industry also avoids the product sensory and chemical differences due to the quality of water source because water must be added to milk powders for reconstitution.

RO concentrates of milk contain all of the milk nutrients in the same proportion as in milk taken for processing. Therefore, RO concentrates of milk can be used as an alternative to milk powders. The RO concentrates can be reconstituted to their original concentration of milk by addition of water; therefore, the RO concentrates of milk act as substitutes for fresh milk or milk powder.

Liquid milk protein concentrate (LMPC) made by UF/diafiltration of skim milk contains 50% to 85% protein on a dry matter basis. LMPC contains whey proteins and caseins in the same proportion as in milk used for its manufacture. LMPC is used in the baking industry for all applications in which milk proteins are used. The usual ratio of caseins:whey protein in LMPC made by MF is 90 : 10. Therefore, LMPC made by MF can be used in all applications in the baking industry where casein proteins are needed. Caseins are rich in lysine and most cereal proteins are deficient in lysine; therefore, milk proteins, especially caseins, enhance the nutritive value of cereals. The protein efficiency ratio of white wheat flour is 1.1 compared to 2.5 for casein, and on blending casein and whey protein to give a mixture containing 75% wheat protein and 25% casein, the PER is increased to about 1.8 (Mulvihill and Ennis, 2003). Milk protein products such as LMPC can be used for water-binding applications for dough consistency. The LPMC are finding increased use in breakfast cereals, milk biscuits, and protein-enriched breads. They can also be used as an emulsifier and texture improver in frozen baked cakes and cookies.

Applications in Cheese

The use of condensed skim in reduced-fat cheese making was reported by Anderson et al. (1993) and McGregor and White (1990). Shakeel-ur-Rehman et al. (2003) reported the manufacture of reduced-fat cheddar cheese from a mixture of cream and liquid milk protein concentrate with high yields. Use of concentrated milks to standardize cheese milk for high yields and throughput has become increasingly important in many large cheese plants that can afford membrane systems. Papadatos et al. (2003) reported an economic advantage of using MF milk in cheese making compared to fortification with NDM or condensed skim milk.

Note: Some of the information in this chapter has been derived from Chapter 13, "Evaporated and Sweetened Condensed Milks," published in *Dairy Processing and Quality Assurance* (Wiley-Blackwell, 2008).

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Chapter 6

Dry Milk Ingredients

Mary Ann Augustin and Phillip Terence Clarke

Introduction

Milk and milk products are supplied as fresh milk products, concentrates, and dry products. Milk powders may be used as a substitute for fresh milk and concentrates. Converting liquid dairy streams into powder provides a supply of milk solids in a convenient, stable form. Dried dairy products are produced by dehydrating liquid milk streams or fractions of dairy streams. Skim milk and full-cream milk powders enabled the development of the recombined milk and milk product industry, which began in the middle of the 20th century and is now well established, with a turnover of more than $5 billion to $6 billion in 2002 (Sanderson, 2004). A number of other powders such as whey and whey protein concentrate powders, protein powders (milk protein concentrates, caseins and caseinates, whey protein isolates), buttermilk powders, and cream powders are available for both the recombined dairy industry and the wider food industry.

The milk powders can be made into a range of reconstituted and recombined dairy products including recombined pasteurized and ultra-high-temperature (UHT) treated milks, in-can sterilized concentrated milk, sweetened condensed milk, cream, ice-cream, fresh cheese, yogurt, and dairy desserts. Milk powders are also used as ingredients in many manufactured food products, in which the components of the milk products (e.g., fat, protein, lactose, milk salts) contribute to the desired properties of the food product.

Milk powders can play many functional roles when incorporated into food products. These have traditionally nutritional roles, because milk is a good source of nutrients, and physical functional roles, because milk powder imparts texture and contributes to the sensory appeal of the final food product. More recently, with the development of the functional food industry and the recognition that milk contains a number of bioactive components, users of milk ingredients are also interested in the physiological role of milk ingredients in manufactured food products. Milk powders also can serve as delivery vehicles for bioactive ingredients.

Developments in milk powder technology and a better understanding of the physical and chemical changes to milk as water is removed has led to improved consistency of milk powders and allowed differentiation of milk powder properties. Different aspects of milk powder manufacture and their applications have been discussed by various authors (Singh and Newstead, 1992; Tong, 2001; Kelly et al., 2003; Augustin et al., 2003; Augustin and Margetts, 2003; Kelly, 2006). The technology of milk powder manufacture and the properties of milk powders and their applications are discussed below.

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Milk Powder Processing

The unit processes in the manufacture of dry milk products include standardization of the milk streams, preheating, concentration, homogenization, and drying. The generalised processes for the manufacture of a selected range of dry milk products, starting with full-cream milk as the raw material, are given in Figure 6.1. The approximate compositions of the major traditional milk powder products are as follows: skim milk powder (36% protein, less than 1% fat, 51% lactose, 8% ash, 3% to 4% moisture) and full-cream milk powder (26% protein, 27% fat, 38% lactose, 6% ash, moisture 3%).

The normal process for the manufacture of full-cream milk powder involves a preheat treatment of the full-cream milk, followed by thermal evaporation, homogenization, and spray drying. An alternative process involves the separation of full-cream milk into skim milk and cream followed by separate heat treatment of each of these streams. The skim milk is given a low-heat treatment and concentrated by evaporation, whereas the cream is subjected to a high-heat treatment. The skim concentrate and cream are then combined back into a full-cream milk concentrate and spray dried. Full-cream milk powders produced by the alternative processes have similar flavor and physical characteristics to powders prepared by the traditional process. Fouling of the evaporators is expected to be reduced with the use of the alternative process (Hols and Van Mil, 1991).

It is essential that milk powders be made from good-quality milk and that their compositional and technical specifications relate to end-use requirements (Jensen, 1990).

Standardization of Dairy Streams

The standardization of dairy streams is essential to ensure that the dry milk products meet

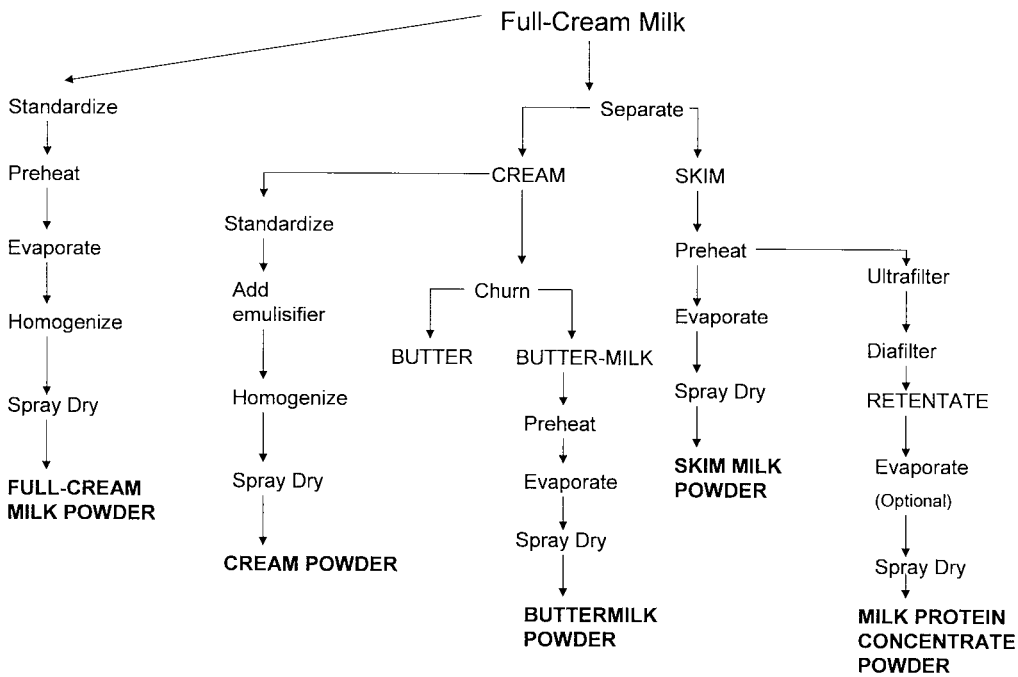

Figure 6.1. Flow chart for manufacture of selected dry milk products.

their required compositional specifications. Traditionally, skim milk powder has been defined as the product resulting from the removal of fat and water in milk that contains not more than 5% moisture and 1.5% fat. This definition requires the lactose, milk proteins, and milk minerals to be in the same relative proportion as in the fresh milk (ADPI, 2002).

With the advent of legislation that allows for standardization of skim milk powder, some specifications now stipulate a level for the minimum protein content. Milk products that can be used for standardizing the protein content of milk include (Codex Alimentarius, 1999):

- Milk retentate, obtained by concentrating milk, partly skimmed milk, or skim milk by ultrafiltration;
- Milk permeate, obtained by using ultrafiltration to remove milk proteins and milk fat from milk, partly skimmed milk, or skim milk
- Lactose

Incorporating protein content into the specifications for milk powders is a logical extension of traditional milk powder specifications because protein has a major influence on the attributes of the milk powder in many applications. Full-cream is standardized to a desired solids-not-fat (SNF) : fat ratio to obtain powder that meets a minimum of 26% milk fat. Buttermilk powder is obtained by drying buttermilk, a by-product of butter manufacture. It should have a minimum protein content of 30% and a fat content greater than 4.5% (ADPI, 2002). One of the distinguishing features of buttermilk powder compared to skim and full-cream milk powders is its high amount of phospholipids. Buttermilk powder also has a stronger dairy flavor.

Specifications for dry milk products, including some additional quality factors, are given in Table 6.1. Although milk powder is a microbiologically stable product, the microbial quality of the raw milk influences the shelf-stability of the powder and it is essential to use good-quality milk to manufacture powders.

Preheating

Milk streams are given a heat treatment prior to concentration, which is essential to obtain dry milk products with good microbiological quality. The heat treatments applied to skim milk vary, ranging from a low heat (typically 72°C [161.6°F] for 15 seconds), to medium heat (75°C [167°F] for 1 to 3 minutes, 85°C to 105°C [185°F to 221°F] for 1 to 2 minutes), to high heat (85°C [185°F] for 30 minutes, 90°C [194°F] for 10 minutes, 120°C to 135°C [248°F to 275°F] for 1 to 2 minutes).

The preheat treatments are also used to achieve a desired level of denaturation of the whey proteins. The heat treatment applied prior to concentration is the prime determinant of the extent of whey protein denaturation during skim milk powder manufacture, because minimal heat damage occurs during the concentration and drying stage (Singh and Newstead, 1992). The extent of whey protein denaturation has a significant influence on the physical functionality of the milk powder in its end use, which has led to the development of a range of indices for classifying skim milk powders based on heat treatment.

The whey protein nitrogen index (WPNI) has traditionally been used as the indicator of the heat treatment used in skim milk powder manufacture (Table 6.2). However seasonal and other natural variations in the protein composition (i.e. whey protein and casein content) of the raw milk supply can affect the WPNI data. Specifically, a fixed heat treatment can result in different extents of whey protein denaturation in milk when there are differences in the amount of whey protein in the raw milk or a change in the ratio of casein:whey protein (Jensen, 1990).

Table 6.1. Specifications for dry milk products.

Product	ADPI[a]	Codex Alimentarius
Skim milk powder		
Fat	Max. 1.25%	Max. 1.5%
Water	Max. 4.0%	Max. 5.0%
Protein		Min. 34%[c]
Titratable acidity	Max. 0.15%	Max. 18[b]
Solubility index	Max. 1.2 ml	Max. 1.0 ml
Bacterial estimate[c]	Max. 10,000/g	
Scorched particles	Max. Disc B (15.0 mg)	Max. Disc B
Full-cream milk powder		
Fat	Min. 26%, Max. 40%	Min. 26%, Max. 42%
Water	Max. 4.5%[d]	Max. 5.0%
Protein		Min. 34%[e]
Titratable acidity	Max. 0.15%	Max. 18[b]
Solubility index	Max. 1.0 ml	Max. 1.0 ml
Bacterial estimate	Max. 10,000/g	
Scorched particles	Max. Disc B (15.0 mg)	Max. Disc B
Buttermilk powder		
Fat	Min. 4.5%	
Water	Max. 4.0%	
Protein	Min. 30%	
Titratable acidity	0.10%–0.18%	
Solubility index	Max. 1.25 ml	
Bacterial estimate[e]	Max. 20,000/g	
Scorched particles	Max. Disc B (15.0 mg)	
Cream powder		
Fat		Min. 42%
Water		Max. 5.0%
Protein		Min. 34%[c]

[a]ADPI (2002) specifications for Extra Grade
[b]As ml 0.1 N NaOH/10 g solids-not-fat
[c]With coliforms not greater than 10/g
[d]As determined by weight of moisture on a milk-solids-not-fat basis
[e]As a proportion of milk solids-not-fat

Table 6.2. Heat classification of skim milk powder.

Milk powder class	Whey protein nitrogen index	Cysteine number[b]	Thiol number[b]	Heat number[b]
Extra-low heat		24–31		
Low heat	≥6	31–38	≤7.5	≤80
Medium heat	1.51–5.99	38–62	7.5–13.3	80.1–88
High heat	≤1.5	≥62	≥13.3	≥88.1

[a]From *Standards for Grades of Dry Milks Including Methods of Analysis,* American Dry Products Institute, Bulletin 916 (2002)
[b]IDF specifications, Wilcek (1990)

Other indicators of the extent of whey protein denaturation, such as the casein number, heat number, thiol number, cysteine number, and furosine content (Wilcek, 1990; Tong, 2001), and sulphydryl content and absorbance at 340 nm (Guingamp et al., 1999) have been also suggested as alternative measures of the extent of heat treatment for skim milk powders (Table 6.2). However, the WPNI method remains the guide that many manufacturers use for classifying skim milk powders.

Full-cream, buttermilk, and cream powders are not generally given a heat classification. Nevertheless, the trends in WPNI relate to the intensity of the heat treatment. High-heat treatments have been shown to extend the shelf life of full-cream milk powder (Baldwin and Ackland, 1991). The treatment inactivates lipase present in milk and develops the natural antioxidant activity of the milk components. Heat treatment causes exposure of the sulphydryl groups and the Maillard reaction; both of these events contribute to an increased oxidative stability of the milk powder (Taylor and Richardson, 1980; McGookin and Augustin, 1997).

Concentration

In the production of skim and full-cream milk powders, the milk stream is thermally concentrated, typically to 45% to 50% total solids, prior to drying in a spray dryer. A multi-stage falling film evaporator is normally used, and the evaporation is performed under vacuum. There is little additional heat damage during concentration after the preheat treatment of the milk (Oldfield et al., 2005) because the residence times in each stage of the evaporator are short (approximately 60 seconds). The maximum temperature the milk is exposed to is 72°C (161.6°F) in the first stage, with lower temperatures in subsequent stages (Singh and Newstead, 1992). Because the thermal evaporation of water is cheaper than removing water by spray drying, it is desirable to feed concentrates with high total solids content into the dryer.

The viscosity of concentrates fed into the dryer influences the properties of milk powder; in particular, increasing viscosity leads to a significant reduction in the solubility of the powder (Baldwin et al., 1980). High viscosity affects the efficiency of the drying process (Bloore and Boag, 1982). Hence, viscosity must be controlled and monitored. The viscosity of the concentrate increases with a higher degree of concentration and a longer holding time of the concentrate. The viscosity of the concentrate is affected by the natural variations in milk composition and by the heat treatment applied prior to concentration.

A high protein concentration in milk can significantly increase the viscosity of the concentrate (Bloore and Boag, 1981). Increasing heat treatment, resulting in a higher extent of whey protein denaturation, also increases the viscosity of the concentrate. Recent work on reconstituted whole milk concentrates confirmed that solids content, heating, and storage temperature affected their rheological behavior, with Newtonian behavior observed at lower total solids and non-Newtonian behavior occurring at higher total solids and higher heating temperatures promoting non-Newtonian behavior at lower solids (Binh et al., 2007).

It has been suggested that the total solids of the concentrate can be increased to greater than 50% when milks are exposed to low- or medium-heat treatments prior to concentration, but should not exceed 50% when high-heat treatment of milk is applied (Vilder de and Moermans, 1983). However, others have demonstrated that increasing the solids content can pose potential problems. Jensen and Hansen (1974) found that although increasing the solids of full-cream milk concentrates from 43% to 49% did not affect the initial solubility of the powder produced, the loss in solubility was more pronounced during 12 months of storage at 30°C (86°F) when concentrates had a higher content of solids.

The initial pH of single-strength milk is pH 6.7 (i.e., the natural pH of milk) and it decreases on concentration. Concentrated milk at 20% total solids has a pH of approximately 6.45. Further concentration to 45% total solids reduces pH even further, and at this concentration the pH is approximately 0.5 to 0.6 units lower than the pH of single-strength milk (Bienvenue et al., 2003). This

is primarily because of the change in the mineral salt equilibria of milk because the milk is concentrated by water removal. Concentration causes transfer of calcium and phosphate to the colloidal phase of milk (Graet le and Brule, 1982), causing a re-establishment of the mineral salt equilibria with the release of hydrogen ions. The high solids and the lower pH of evaporated milk concentrates make them more susceptible to aggregation.

Milks also may be concentrated by membrane processing. This method of concentration is used for the production of milk protein concentrates (Figure 6.1). In contrast to the removal of water only by thermal evaporation, concentration using membranes results in the fractionation of the milk's components. The partitioning of the milk components depends on many factors including the size of the membranes, the extent of ultrafiltration and diafiltration, and the conditions (e.g., pH of feed, temperature) used for these separation processes. Membrane processing leads to concentration of the milk protein in the retentate streams which are subsequently dried to produce milk powders with higher protein content than that of traditional skim milk powder.

Milk protein concentrate (MPC) powders with varying protein contents (40% to 85% protein) have been made by ultrafiltration and/or diafiltration or evaporation of the ultrafiltered retentate prior to drying. Milk protein concentrate powders may be made as low- or high-heat products (Getler et al., 1997; Huffman and Harper, 1999). The total solids of the concentrate that can be fed into the dryer depends on the protein content of the MPC produced, but is much lower than that used in the production of skim milk powders. The high-protein and low-lactose content of the concentrates prepared by ultrafiltration/diafiltration leads to the higher viscosity, which limits the solids content of the concentrates that can be fed into the dryer.

Homogenization

Homogenization of the full-cream milk concentrate prior to spray drying is a routine step in the traditional process for manufacture of full-cream milk powder (Figure 6.1). During homogenization, the milk concentrate is fed under high pressure through a small orifice which disrupts the native milk fat globule. The size of the globule is reduced, and this is accompanied by an increase in the surface area of the fat. The natural milk fat globule membrane is insufficient to cover the increased area and a new membrane is formed, comprising a mixture of the original milk fat globule membrane and adsorbed milk proteins.

The purpose of homogenization is to decrease the surface free fat in the milk powder (Vilder de et al., 1979). Generally, full-cream milk powders are manufactured to obtain a low level of free fat in powder. This is because a high surface free fat (above 2% of powder) in full-cream milk powders is undesirable because flowability, wettability, and storage stability of the powder are adversely affected.

Drying

In the drying stage, dryers remove water from a milk concentrate to produce a shelf-stable product. Early commercial milk powder drying plants used roller dryers. The concentrate was fed over rotating steam- or oil-heated drums to evaporate the water from the concentrate. The resultant sheet of powder was then powdered in a hammer mill to a predetermined size. The powders produced have sharp edges and are made up of compact particles (Caric and Kalab, 1987). Except for the manufacture of full-cream milk powder with high free fat content for the chocolate industry, today most industrial milk powder plants use spray dryers (Figure 6.2).

In spray drying, milk concentrates are fed into dryers with positive displacement pumps.

Figure 6.2. Spray dryer.

The concentrate is atomized, using a rotary atomizer or a nozzle, to obtain small droplets of concentrate. Water is rapidly evaporated from the droplet surface when it is initially mixed with the hot air in the drying chamber. The resultant dried powder particles are separated from the drying air by cyclones, collected, and packaged. Spray-dried milk powders have a globular shape, with a convoluted surface and a porous structure. The method of atomization used affects the microstructure of the powder, with nozzle atomization resulting in lower occluded air and higher powder bulk density (Caric and Kalab, 1987; Tong, 2001).

Although the inlet air temperature of the dryer is high (above 170°C; 306°F), there is minimal heat damage to proteins during spray drying because of evaporative cooling as water is removed rapidly and the particle is dried. The temperature of the particle is low (below 70°C; 158°F) until the milk powder droplet is dried. The outlet air temperature of the dryer has the greatest effect on heat damage because this is the temperature the dried powder particle approaches (Singh and Newstead, 1992). Lactosylation of milk proteins, which is the conjugation of lactose to protein and is related to heat damage, can occur during skim milk powder manufacture. It is promoted with the use of high outlet air temperatures during drying (Guyomarc'h et al., 2000).

The operating conditions of spray dryers can affect the solubility characteristics of milk powders. The detrimental effects of high-temperature drying conditions are more pronounced during manufacture of high-protein milk powders compared to standard skim and full-cream milk powders. In the

manufacture of milk protein concentrate powders (75% protein in powder), impaired hydration properties were obtained when the outlet temperature of the spray dyer was increased from 65°C (149°F) to 95°C (203°F) (at a constant inlet temperature of 250°C; 482°F). Detrimental effects on hydration properties of MPC powders were also obtained with an increase in the inlet temperature (Castro de and Harper, 2001; Castro-Morel de and Harper, 2003).

A number of comprehensive descriptions of the engineering aspects of spray drying are available (Masters, 1991 and 2002; Mujumdar, 1995; Pisecky, 1997). Further developments in process control techniques for optimization of milk powder production require an improved understanding of the thermodynamic properties of the milk and concentrate and the interactive effects of time, temperature, and shear during the course of milk powder manufacture (O'Callaghan and Cunningham, 2005).

Instantization

Instantization is carried out to improve wettability, dispersibility, and the free-flow properties of milk powders. Instantizing may be achieved by returning the fine powder particles into the dryer close under the rotary atomizer or spray nozzles so that the particles aggregate to form agglomerated powder.

Lecithin is often used to improve the properties of instantized milk powders (Sanderson, 1978). A traditional method of application involves dissolving the lecithin in butter oil and spraying over the agglomerated milk powder, either internally or in a fluidized bed external to the dryer. The process requires strict adherence to controlled temperatures during powder manufacture to allow both the hydrophilic and lipophilic components of the lecithin to interact with the free fat in a molten state. Hence, when using a wetting agent such as 30% to 50% lecithin in an oil solvent, the mixture should be 60°C to 65°C (140°F to 149°F), the powder temperature must be a minimum of 50°C (122°F), and the powder must be fluidized for at least 5 minutes at 45°C (113°F) (Pisecky, 1997).

Recently an alternative *in-situ* process for lecithination of skim milk powders has been reported. In this process lecithin is added to the feed introduced into the spray dryer. This approach was based on the finding that the surface of spray-dried milk powder is dominated by surface-active species and that the most rapidly adsorbing surface-active agent dominates the composition of the surface of a powder particle (Millqvist-Fureby and Smith, 2007).

Properties of Milk Powders

Powders in the market must meet general standard specifications for trade (Table 6.1). This is the minimum requirement. The composition and microbiological quality of the milk powder, though essential attributes, do not always reflect their performance in their intended application. A number of tests have been developed for further characterization of milk powders. These may be used as quality control measures or as a guide for assessing the physical functionality of the powders.

Physical Characteristics of Powders

The physical characteristics of milk powders must be understood if they are to be fit for the intended application.

Bulk Density

Bulk density is a measure of the weight of powder that can be contained in a set or known volume. It is also referred to as packing density and can be expressed in g/cm^3, kg/m^3, or g/100 ml (Pisecky, 1997). The bulk density is a consideration for packaging, particularly when transport is being costed on a volume basis. Bulk density also has an

influence on other aspects of powder functionality including dispersibility, wettability, and instantizing.

The bulk density of milk powders is measured on a known weight of powder transferred to a measuring cylinder. The initial volume is the poured bulk density. The cylinder is then tapped, usually 100 times, and then a further 525 times to give the loose and final bulk density, respectively. There are also variations on this number of taps. There are many manual (ADPI, 2002) and automated (e.g., Stampvolumeter) methods for this determination, and the method must always be quoted when giving determinations. Typical results for skim milk powder are in the range of 0.58 to 0.68 g/ml. For full-cream milk powder, the bulk density is 0.56 to 0.66 g/ml for nonagglomerated powder and 0.45 to 0.52 g/ml for instantized powder.

During powder manufacture many variables can play a part in the final bulk density of the powder produced including the concentrate characteristics, atomization methodology, drying parameters, and extent of whey protein denaturation. The parameters that determine the final bulk density are the occluded air (i.e., the amount of air entrapped within the individual powder particles), interstitial air (i.e., the amount of air or space between the powder particles themselves), and distribution of the size and shape of the powder particles. Powders with a range of particle sizes give a higher bulk density than those with a narrow particle size distribution. With respect to the effects of shape, powder particles that are smoother and more uniform give rise to higher bulk densities.

Flowability

Powder flowability is an important attribute of milk powders in the area of transport, packaging, and handling. The measurement of flowability is particularly difficult. Measurements can be carried out using one of the sophisticated analytical instruments on the market (e.g., Hosakawa micron powders tester, Aeroflow powder flowability analyzer) or simple tests involving measurement of powder flow through a funnel, down an incline, or the angle of repose after forming a powder pile under controlled conditions. An alternative method uses a rotating drum developed by Niro, and this gives results for a wide range of powders (Piesecky, 1997). Measurements using this drum method indicate that the flowability of agglomerated skim milk powder is greater than that of agglomerated full-cream milk powder, which is greater than that of instant full-cream milk powder, which is greater than the flowability of ordinary full-cream milk powder.

Flowability is a very complex issue that is influenced by many factors. It is improved by the use of flow additives, minimizing the amount of fines, increasing the particle size, and having more spherical and smooth particles. An increase in moisture, particularly surface moisture and/or fat content, particularly free fat, has a detrimental effect on flowability.

Reconsitutability

The ability of a powder to be reconstituted depends on its ability to be wet, to sink, to disperse, and finally to dissolve. Complete dissolution is important for functionality of powders in an application.

Wettability: In order for a powder to be reconstituted it must first be penetrated by the water in which it is being dissolved. The powder must be able to overcome the surface tension between itself and the water in the first instance.

A typical method for measuring wettability consists of systematically placing a weighed amount of powder on the surface of a known volume of water at a set temperature and then measuring the time taken for all of the powder to disappear below the surface of the water (Pisecky, 1997).

The degree of wettability is strongly influenced by several factors; two of the most significant are the free fat content of the powder and the state of the lactose. Under some conditions of manufacture or storage, the amorphous lactose may be changed to a crystalline state and damage the fat globule membrane (Kelly et al., 2003), causing an increased level of free fat in full-cream milk powders. One way to overcome the problem associated with reduced wettability is to add surfactants such as lecithin to the powder. Wettability is also reduced when there is an increase in interstitial air within the powder particles.

Sinkability: A closely aligned attribute to wettability is the sinkability of powders. Once the powder particle has been initially wetted it then must be able to sink into the water for complete dispersion and solubility.

Sinkability may be measured by recording the time required for the disappearance of powder from the water surface after a portion of a milk powder has been added to water and stirred with an impeller under fixed conditions (Schober and Fitzpatrick, 2005).

The conditions used for reconstitution influence the sinkability of a powder. The creation of a vortex and maintaining it during reconstitution is crucial for sinkability. The particle density is also an influencing factor in sinkability in that the heavier the particle/unit volume the more likely it is to sink. Thus, low interstitial air content is a prerequisite to good sinkability.

Dispersibility: During the process of dissolving powders the agglomerates need to instantly disintegrate into single particles to facilitate wettability and dissolution.

The dispersibility of powders is measured by systematically placing a weighed amount of powder (typically 10 g) onto the surface of a set amount of water (250 ml at 25°C; 77°F), stirring the solution for a set time in a rotational pattern, sieving the contents, and after drying, weighing the residue. The dispersibility is reported in terms of the mass of the test portion and the values for water content and total solids (Pisecky, 1997).

To facilitate dispersibility, the agglomeration process must be controlled to produce few if any agglomerates exceed 250 microns in size.

White flecks: White flecks are particles that remain undissolved in a milk solution after reconstitution. They can be observed when the solution is spread to form a thin film, for example, on the back of a spoon after the solution has been allowed to stand for several minutes. The white flecks can also form a surface layer. They tend to be more prevalent when high total solids solutions are prepared. Although white flecks tend to be rather soft, they can cause physical problems when the reconstituted milk is used in processing operations, because they can clog filters and sieves and can be visibly undesirable in the final product.

Physical Functionality of Powders

When a milk powder is used in an application, either as the primary or secondary ingredient, it imparts physical attributes to the final products which are often essential for the success of the application. These physical attributes include solubility, heat stability, gel forming, thickening or viscosity control, foaming, and binding characteristics.

Solubility

Solubility is a prerequisite for most other functional attributes because if the powder cannot be efficiently solubilized then it cannot impart the desired attribute effectively. If the powder is not completely dissolved it can cause problems in processing such as clogging of filters and losses of material due to sedimentation. There is also the need for subsequent removal of undissolved material.

Powders are tested for insolubility by determining the amount of insoluble material remaining after a prescribed method for dispersion of the powder at a nominated total solids concentration at defined temperature and mixing techniques. The most common of these is the method used by the American Dry Products Institute (ADPI) (ADPI, 2002). The insoluble material is usually made up of denatured protein (typically β-lactoglobulin) complexed with casein and lactose in various ratios.

A range of factors are known to contribute to the formation of insoluble material in milk powder. The most critical factor controlling the insolubility of powders is the temperature of the particle during the removal of water in the dryer when the moisture content is between 10% and 30%. Other factors that contribute to insolubility include the preheat treatment of the milk during manufacture, when higher temperatures more often lead to higher insolubility, type of dryer used (roller dryers are worse than spray dryers), configuration of the spray dryer (such as the type of atomization) and single-stage versus multi-stage drying, and the physical properties of the concentrate prior to drying (e.g., viscosity).

Another very critical factor that influences powder solubility is the temperature at which the milk powder is reconstituted. Solubility is usually highest between 40°C and 60°C (104°F and 140°F), particularly when preparing a high-solids reconstituted concentrate from powder.

Heat Stability

Heat is used in some form during the processing of most products. Therefore, milks reconstituted from powders, when incorporated into a product, are subjected to heat of various degrees. During heating the milk is required to not unduly thicken or coagulate, depending on the application. The susceptibility to heat is magnified in concentrated milk solutions such as evaporated milk.

Several alternative methods have been developed in an attempt to measure heat stability of powders in general as well as for specific end uses. Typical of the common methods is measuring the coagulation time of a milk solution at a specific total solids, at temperatures in the range of 120°C to 140°C (248°F to 284°F). Another method using time coagulation criteria is the ethanol stability test (Horne and Parker, 1980) in which mixtures of reconstituted milk with various amounts of ethanol are used. The drawback of these tests is that they measure the heat stability of the milk solution under defined conditions, which do not directly predict the heat stability in the intended application in which the environment can be quite different.

The closer the conditions of the test are to the conditions used in the intended application, the better the correlation. An objective laboratory-scale method for examining the suitability of skim milk powders for recombined evaporated milk manufacture has been developed. The method involves heating concentrated milk to 120°C (248°F) for 13 minutes and measuring the viscosity of the sterilized concentrated milk. This method has proven to be a good guide to the stability of recombined evaporated milk during retorting (Kieseker and Aitken, 1988). This laboratory-based method is an alternative to costly and time-consuming pilot plant trials.

Many factors affect the heat stability of milk powders. Heat treatment of milk applied during powder manufacture has been used to manipulate the heat stability of milk powders. A high-heat treatment of milk, which results in a high level of whey protein denaturation (i.e., a low whey protein nitrogen index, WPNI less than 1.5 mg denatured whey protein/g powder), is desired for adequate heat stability of concentrated milks. However, reliance solely on WPNI to assess the ability

of concentrated milks to withstand subsequent heat treatment is not recommended because other factors (e.g. pH, mineral balance of milk) can have a more significant effect on heat stability.

Viscosity

Milk powder is used to influence the viscosity of products in a range of applications. Viscosity control is particularly important in high-solids products such as recombined sweetened condensed milk.

The viscosity of milks reconstituted from milk powders is usually measured by a method aligned with the application in which the powder is intended. A single-strength solution at a specified temperature is a good starting point for many applications. However, in applications in which a higher-than-single-strength solution is to be used, then specific tests must be undertaken that mimic the environment of the application. For example, tests for assessing the suitability of milk powders for recombined sweetened condensed milk involve making a mixture of skim milk powder, sugar, and water in the same proportions as the final product; heating the mixture under standardized conditions that are representative of the process used in industry; and measuring the viscosity of the final mixture (Kieseker and Southby, 1965; Weerstra et al., 1988). Alternatively, pilot scale trials can also be carried out to test suitability of powders for recombined sweetened condensed milk applications.

The major factor that influences the viscosity of recombined sweetened condensed milk is the preheat treatment of the skim milk applied during powder manufacture. Generally, medium-heat milk powders are suitable for this application. Increasing the extent of whey protein denaturation in the powder to higher than 50% results in marked increases in viscosity of recombined sweetened condensed milk (Cheng et al., 2000).

Gelling

Milks do not gel at their natural pH. However, they gel on acidification as the pH is reduced to pH 4.6. Gelation is a consequence of the reduction of the charges on the milk proteins as the pH approaches the isoelectronic point. Acid milk gels may be formed by addition of an acidulant such as glucono-delta-lactone or with the use of cultures as in the production of yogurt. The strength of milk gels may be measured using standard texture analyzers (e.g., Instron, TA-XT2 texture analyzer).

The acid gelation properties of milk are affected by the milk composition and the heat treatment applied during powder manufacture. Firmer gels are made with milks that have been given a high heat treatment. Improved yogurt properties are obtained with increasing whey protein denaturation in milk powder (Augustin et al., 1999).

Foaming and Emulsifying Properties

Milk powders offer good emulsifying and foaming capabilities that are required for some applications. In skim milk powder, the main surface-active components are the milk proteins, whereas in full-cream milk powders, there is also the phospholipid component of the milk fat globule membrane. Caseins, whey proteins, and phospholipids are able to stabilize the air-water interface of air bubbles in foams and the oil-water interfaces of fat droplets in emulsions due to their amphiphilic properties.

Foaming capacity can be measured by simple methods using domestic mixers with milk solutions at set times and temperatures and measuring the resultant foam generated (Phillips et al., 1987). The emulsion capacity of milk powder solution can be determined by the principle of pumping oil into a protein solution while homogenizing and monitoring the electrical resistance of the solution. A decrease in electrical resistance is observed when the solution changes from an oil-in-water emulsion in which water is the continu-

ous phase to a water-in-oil emulsion with oil as the continuous phase. A typical example of this method is that by Vuillemard et al. (1990).

The foaming and emulsifying properties of milk powders can be influenced by their composition and processing treatments applied to the milk as well as the conditions used for the formation of emulsions and foams. Physical changes can be made to the powder morphology to enhance the foaming capacity. This may be done by manufacture of high occluded air in powders by altering processing variables or by injecting air into the concentrate prior to drying.

Storage Stability of Milk Powders

The physical properties of milk powders may be altered when they are stored. Storing powders at high temperature and humidity accelerates the damage to milk powders. A number of phenomena and reactions cause the quality of milk powders to deteriorate, including lactose crystallization chemical and enzymic reactions. The consequences of these reactions are a loss of solubility and impairment in many of the other physical (e.g., increased tendency to cake and reduced flowability) and functional attributes (e.g., gelling, emulsifying, and foaming) of milk powders. The effects of aging on the properties of milk powders have been reviewed (Thomas et al., 2004).

Applications of Milk Powders

Milk powders are used in a range of applications. The physical functional attributes of powders govern their ability to contribute to attributes of the final product. Figure 6.3 depicts the road map for using milk powders. Success in using milk powders requires an understanding of the properties of the individual powder ingredients and how their functionality is expressed in the final food product. This is because the milk powder components can interact with other ingredients when formulated and processed into a final recombined dairy product or food product. For the purpose of quality control, it is beneficial for milk powder suppliers to work with end users of their ingredients and understand how their products will be formulated and processed. This allows the development of more appropriate fitness-for-purpose specifications and methods for testing the attributes of milk powder ingredients.

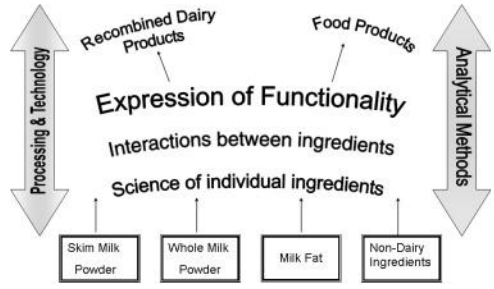

Figure 6.3. Road map for using milk powders.

Milk powders can also impact flavor and color of products. Buttermilk is sometimes used as a partial replacer of other milk powders to improve the flavor of dairy products. In some applications, a bland milk powder is desirable so as not to impart flavor tones which may be undesirable for the specific product.

Recombined and Reconstituted Dairy Products

Early reconstitution and recombination was for the manufacture of simple products such as liquid milk, sweetened condensed milk, and evaporated milk. Today all traditional milk products can be made from milk powder and milk-based ingredients. Figure 6.4 shows the operations involved in the process required for the production of recombined milk.

Reconstitution is simply the mixing of milk powder with water. For example, reconstituted skim milk and reconstituted

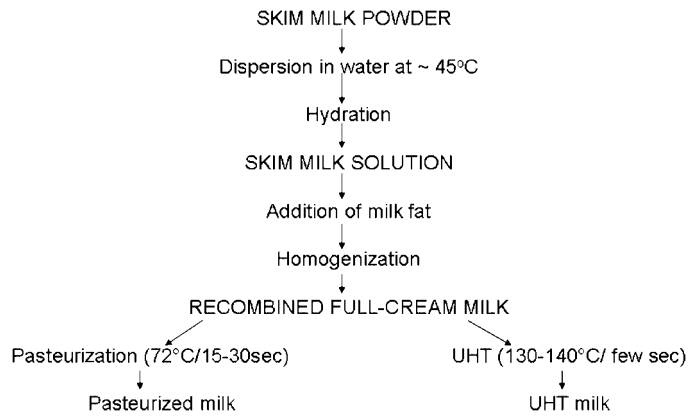

Figure 6.4. The recombination of milk process.

full-cream milk are made by dispersion of a skim milk powder and full-cream milk powder in water. Depending on the shelf-life required, the reconstituted milk may be pasteurized or given a high-heat treatment for sterilization. When several milk ingredients are mixed for the production of a dairy product, this process is termed recombining. In this case, usually the skim milk powder ingredient is reconstituted in water and combined with anhydrous milk fat, homogenized, and heat treated.

Typical recombined milk products include recombined UHT single-strength milk (e.g., 12% total solids, 9% skim milk solids : 3.5% fat), recombined retort-sterilized evaporated milk (e.g. 26% total solids, 18% skim milk solids : 8% fat), and recombined sweetened condensed milk (e.g., 74% total solids, 20% skim milk solids : 8% fat : 46% sucrose). Other recombined dairy products include recombined cheese, cultured milk products (e.g., labneh, yogurt), cream, and ice-cream (Jensen, 1990). When the milk fat component in a recombined dairy product is substituted with a non-dairy fat (e.g., canola oil, palm oil), the resultant product is called a filled dairy product.

Milk powders can now be tailored to suit a vast range of applications for the manufacture of dairy products because milk powder processes can manipulate composition, physical, and functional characteristics. However, the heat treatment given during skim milk powder manufacture remains the dominant factor for selection of powder for a particular end use in a manufactured recombined dairy product (Table 6.3). Nevertheless, other characteristics (e.g., quality of the powder) also need to be considered.

Applications in Non-dairy Products

A growing number of non-dairy products are manufactured using milk powders as an ingredient. Milk powders are found in such products as meat, bakery, confectionery, chocolate, sauces, and desserts. Their functional characteristics, essential in many applications, include browning and flavor development, water binding, emulsification, viscosity modification, and texture. The inherent properties of milk powders may be used as a guide to powder selection for specific end uses (Table 6.3). However, the functionality of a powder in the final application depends on the ingredients in the food formulation and the processing variables used in the manufacture of the end food product.

Table 6.3. Functional requirements of skim milk powders in selected applications.

Product	Heat treatment of milk powder	Desirable powder attributes
Single-strength milk		
Pasteurized milk	Low-medium heat	Reconstitutability Good flavor Emulsifying
UHT milk	Low-medium-high heat	Reconstitutability Good flavor Heat stability Emulsifying Low level of heat-stable enzymes
Concentrated milk products		
Evaporated milk	High heat	Reconstitutability Heat stability Viscosity
Sweetened condensed milk	Low-medium heat	Reconstitutability Viscosity
Other recombined dairy products		
Yogurt	Low heat	Water binding Viscosity Gelling
Cream	Low-medium heat	Good flavor Emulsifying
Cheese	Low heat	Rennetability
Other products		
Ice cream	Low-medium-high heat	Foaming/whipping Emulsifying
Confectionery	High heat	Water binding Foaming/whipping Emulsifying Heat stability
Bakery	High heat	Water binding Foaming/whipping Emulsifying Gelling
Meat products	High heat	Water binding Foaming/whipping Emulsifying Gelling

Specialized Milk Powders

In addition to conventional milk powders, there is a growing range of powders that are specially tailored to provide physical, nutritional, or physiological functional roles in a food product.

Milk Powders for Chocolate Manufacture

When full-cream milk powder is intended for chocolate manufacture, a high level of free fat is desirable to reduce the amount of cocoa butter and surfactants needed in the chocolate formulation as the viscosity of the chocolate mass is reduced and less energy is used for chocolate manufacture. When the traditional process is for manufacture of full-cream milk powder (Figure 6.1) with a typical fat content of 26% fat, a powder with low level of free fat is obtained. However, roller-dried powders have high levels of free fat, making them more suitable for chocolate manufacture (Reimerdes and Mehrens, 1993; Augustin, 2001). Compositional factors can impact the free fat content of milk powder. Higher solid-fat content of the fat (Twomey et al., 2000) or the fat content of the concentrate (Kelly

et al., 2002) can lead to an increase in free fat of spray-dried milk powders.

Significant increases in free fat in powder also may be achieved by modifying the method used for manufacture. Higher free fat powders were obtained by (a) increasing the temperature of the concentrate fed into the spray dryer or decreasing the inlet air temperature and increasing the outlet air temperature of the spray dryer (Vilder de et al., 1976, 1979), (b) combining skim concentrate with cool cream or cream homogenized at a high temperature prior to drying (Clarke and Augustin, 2005), or (c) exposing full-cream milk powder to high shear and a high temperature in a twin-screw co-rotating processor (Koc et al., 2003).

Comparisons between the performance of roller-dried full-cream milk powder, spray-dried with added butter oil, and spray-dried full-cream milk powder with a high free fat content indicated that free fat was a major influence on the rheological properties of chocolate (Franke et al., 2002). Examination of spray-dried milk powders produced by mixing milk fat fractions into skim milk prior to drying, spraying milk fat fractions onto dried powder, or a combination of these showed that there was a good correlation between free fat content of the spray-dried powders and viscosity of chocolate mass, although other factors such as the microstructure and interfacial properties of the powders also had a role (Attaie et al., 2003).

Milk Powders with Health-promoting Functional Ingredients

The interest in the development of health-promoting foods has led to research in functional milk powders for health and well-being. These include milk powders enriched with well-known nutrients such as minerals (e.g., Ca) and vitamins (e.g., vitamins A and D), and functional ingredients of more recent interest such as omega-3 oils, probiotics, and phytosterols. Some of these functional ingredients are added as microencapsulated ingredients, whereas others are directly incorporated into milk powders (Augustin, 2003).

The well established role of calcium in bone health has driven interest in the development of calcium-fortified milk products (Augustin and Williams, 2002). Insoluble calcium salts may be dry blended with milk powders but there are potential problems with separation during powder storage and settling of these salts when used in reconstituted milk applications. Soluble calcium salts may be added, but their addition must be carefully managed to avoid protein precipitation of milk during heating because the direct addition of soluble calcium salts increases calcium activity and reduces the pH of the milk, making it more susceptible to coagulation. A strategy based on the addition of soluble calcium salts in combination with orthophosphates for management of calcium activity and pH control has been applied to produce calcium-fortified milk powders with up to 8 g additional calcium/kg powder. This approach involves fortification of milk followed by a low- or high-heat treatment of milk prior to concentration and drying (Williams et al., 2005).

Probiotics have a role in gut health and have been added to a range of foods. They are generally supplied as freeze-dried cultures for addition to foods. It is possible to produce a probiotic milk powder by spray drying reconstituted skim milk containing *L. paracases* NFBC 338 with a probiotic survival of 85%. It was further demonstrated that the probiotic powder could be added to cheese milk for production of probiotic cheddar cheese (Gardiner et al., 2002).

The incorporation into foods of omega-3 fatty acids has been increasing due to their link with improved heart, eye, and brain function. Spray-dried milk powder enriched with omega-3 oils may be produced from full-cream milk supplemented with a range of omega-3 oils (e.g., fish oil) prior to spray drying. Special care must be taken in produc-

ing omega-3-enriched milk powders because omega-3 oils are susceptible to oxidative deterioration. Omega-3 enriched milk powders containing 2.4% and 2.1% eicosapentaenoic acid (EPA) and dodecahexaenoic acid (DHA), respectively, that were made by supplementing milk with fish oils, were found to be stable for approximately five months (Ramaprasad et al., 2006). An alternative process is to dry blend stabilized microencapsulated omega-3 powders with milk powders, an approach that has been used for the production of omega-3-enriched infant formulas that are currently available.

Phytosterols are added to a variety of foods due to their cholesterol-lowering properties. A potential limiting factor for these compounds is their susceptibility to oxidation, which leads to the formation of undesirable byproducts. However, this was not an issue for a phytosterol-enriched whole milk powder containing 7% phytosterol. A phytosterol-enriched milk powder that was produced by spray drying a concentrated milk emulsion with incorporated microcrystalline phytosterol suspension in fat was stable for 12 months at room temperature or slightly elevated temperatures of 38°C (100.4°F) (Soupas et al., 2006).

Conclusion

Conventional skim and full-cream milk powder products are expected to remain major commodities of the dairy industry. However, the market demands for milk-based powders with enhanced functionality for specific end uses with more stringent functionality requirements will continue to drive the development of differentiated milk powders. The capacity of milk and dairy products to contribute nutritional and physical attributes to food products and have a physiological functional role will no doubt ensure the long-term viability of the milk powder industry. The development of new dairy-based powders relies on continued research into the structure and function of milk components in various environments and how their properties can be manipulated and controlled by the application of conventional and emerging food processing technologies (Augustin and Udabage, 2007).

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Chapter 7

Casein, Caseinates, and Milk Protein Concentrates

Mary Ann Augustin, Christine M. Oliver, and Yacine Hemar

Introduction

Milk is a complex fluid. Typically bovine milk has about 12.5% total solids, the major components of which are the proteins (3.5%), fat (3.8%), and lactose (4.6%). The main proteins in milk are the caseins and the whey proteins. The ratio of casein : whey protein is approximately 4 : 1. Milk also contains mineral salts (0.7%) and many other minor components. Although the mineral salts are minor components, they have a major influence on the functional properties of the milk proteins.

Milk proteins are valued ingredients with high nutritional value and a range of physical functional properties that make them sought-after ingredients for foods. In the dairy industry, milk is often separated to obtain fractions of protein products. The development of milk protein fractions has enabled the dairy industry to diversify its range of protein products. Among some of the differentiated dairy protein products are the caseins and caseinates, whey protein products, such as whey protein concentrates and isolates, and milk protein concentrates, which contain both the casein and the whey proteins in the ratio at which they occur in normal milk.

This chapter discusses the casein-based milk protein products—caseins, caseinates, and milk protein concentrates. The chemistry of casein and the casein micelle, processing methods for manufacture of casein-based milk protein products, their composition, functional properties, and applications in dairy and non-dairy food products are covered.

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Chemistry of Caseins

Caseins are novel, milk-specific, acid-insoluble phosphoproteins that encompass approximately 80% of the total protein in milk. They are readily separated from the other milk proteins (i.e., the whey/serum proteins) by isoelectric precipitation of casein at pH 4.6, 30°C (86°F) (Fox and Kelly 2004). Casein consists of four major protein fractions: α_{s1}-, α_{s2}-, β-, and κ-casein, which have molecular weights between approximately 19 and 25 kDa, and occur in an approximate 4 : 1 : 4 : 1 weight ratio in bovine milk (Fox and Kelly 2004). Fragments of casein, caused by the action of plasmin and which are known as γ-caseins, are also present in milk (Fox and Kelly 2004).

The Individual Caseins

The primary structures of the caseins have been established and numerous genetic variants of each of the caseins have been identified (Farrell et al. 2004). Extensive detail on the physicochemical properties of the individual caseins is reported (Kinsella 1984). Several salient features of the caseins are

Table 7.1. Properties of the major caseins in bovine milk. Adapted from Eigel et al. (1984), Fox and Mulvihill (1983).

Protein	% Total casein	Molecular weight	Phosphate residues (mol/mol protein)	Proline residues (mol/mol protein)	Hydrophobic regions	Sulfhydryl groups
α_{s1}-casein	44–46	22,068–23,724	8–10	17	1–44, 90–113, 132–199	–
α_{s2}-casein	12	25,230	10–13	10	90–120, 160–207	2
β-casein	32–35	23,944–24,092	4–5	35	2/3 of C terminal end	–
κ-casein	8–12	19,007–19,039	1	20	5–65, 105–115	2

provided in Table 7.1, and a select number of these are discussed in further detail below.

β-Casein is the most hydrophobic casein and contains a hydrophobic region that encompasses two-thirds of the protein in the C-terminal end (Farrell et al. 2004). α_{s2}-Casein, the most hydrophilic casein, contains three distinct hydrophobic domains that are distributed along the polypeptide (Farrell et al. 2004). κ-Casein is the only glycosylated casein with complex oligosaccharides, comprising galactose, sialic acid (*N*-acetylneuraminic acid), and galactosamine, which are attached to Thr residues in the C-terminal region. The attachment of the oligosaccharides increases its level of hydrophilicity (Farrell et al. 2004).

A unique feature common to all of the caseins is the presence of phosphate groups. The phosphate groups occur esterified to Ser residues, and to a minor extent, to Thr residues (Fox and Kelly 2004). α_{s2}-Casein is the most phosphorylated, with 10 to 13 phosphoserine residues (Farrell et al. 2004). α_{s1}-Casein and β-casein are extensively phosphorylated, with eight to ten, and four to five phosphoserine residues, respectively (Farrell et al. 2004). κ-Casein is the least phosphorylated with only one phosphoserine (Farrell et al. 2004). The phosphate groups bind calcium and tend to occur in clusters of two to four residues (Holt 1992, Swaisgood 2003).

Owing to their high levels of phosphorylation, α_{s1}-, α_{s2}-, and β-casein are precipitated by calcium concentrations greater than approximately 6 mM at 20°C (68°F), and are referred to as calcium-sensitive caseins (Fox and Kelly 2004). κ-Casein, with just one phosphoserine, is relatively calcium-insensitive and when combined with the calcium-sensitive caseins can stabilize and protect the calcium-sensitive caseins from precipitation via formation of colloidal particles, termed casein micelles.

Caseins are rheomorphic (Holt and Sawyer 1993) due to a high content of proline residues (Farrell et al. 2004) and readily self-associate in solution. For example, α_{s1}-casein can form polymers via end-to-end association of its hydrophobic region (Thurn et al. 1987). β-Casein forms spherical particles in the absence of calcium (Rollema 1992), and κ-casein polymerizes via hydrophobic and intermolecular disulphide bonds (Fox and Kelly 2004). Their lack of intrinsic structure affords stability to the caseins against denaturants such as heat or urea (Fox and Kelly 2004), and also renders the caseins as ideal substrates for enzyme action. For example, caseins are readily hydrolyzed by proteases, which is important for their digestibility and in cheese ripening (Sousa et al. 2001). Hydrolysis of caseins also results in peptides with various bioactive properties (Hartmann and Meisel 2007).

The Casein Micelle

Casein micelles contain approximately 85% to 90% of the caseins in milk (Fox and Kelly 2004). They exist as porous, spherical, micellar aggregates of 50 to 600 nm in diameter (average 100 nm) (de Kruif and Holt 2003).

A typical casein micelle contains approximately 10^4 individual casein molecules (Fox and Kelly 2004). The calcium-binding properties of the caseins and their organization into casein micelles facilitates a high concentration of calcium phosphate to be carried by the caseins, which would otherwise precipitate and block the mammary glands. It is not surprising then that the main physiological function of the casein micelle system is to prevent pathological calcification of the mammary gland (de Kruif and Holt 2003).

The casein micelles are extensively hydrated, with approximately 3.4 g water/g dry matter (Morris et al. 2000). Approximately 15% of the water is bound to the protein (de Kruif and Holt 2003, Farrell et al. 2003) and the remaining water is occluded within the casein micelle. On a dry basis the micelles comprise 94% protein and 6% colloidal calcium phosphate (CCP), which is essentially a mixture of calcium and phosphate with small quantities of magnesium, citrate, and trace metals (e.g., zinc) (McGann et al. 1983). Removal of CCP by acidification or calcium chelating agents disperses the micelles into smaller particles; hence, CCP plays an integral role in the structure of the casein micelles (de Kruif and Holt 2003). Protein-protein interactions including electrostatic and hydrophobic interactions and hydrogen bonding also appear to be involved in maintaining casein micelle integrity (de Kruif and Holt 2003, Horne 1998).

Various models for the casein micelle structure have been suggested and refined over the last 50 years. Recent critical reviews of the various models are available in the literature (for example, see Fox 2003, Fox and Kelly 2004, Horne 2006, Phadungath 2004, Qi 2007). Selected models are described here.

The dual binding model emphasizes the role of hydrophobic interactions between casein molecules in maintaining the integrity of the casein micelle (Horne 1998). An alternate model attributes interactions between the caseins and calcium phosphate as the framework of the casein micelle structure. In the latter model calcium phosphate is in the form of nanoclusters, which interacts with the caseins via the phosphoseryl clusters of the calcium-sensitive caseins (Holt 1992, Holt et al. 2003). More recently, an interlocked lattice model has been proposed in which polymeric casein aggregates and casein-calcium phosphate aggregates contribute to casein micelle integrity (McMahon and Oommen 2008). The polymeric casein aggregates interact via hydrophobic and electrostatic interactions and calcium bridging. These aggregates are believed to be interlocked within the casein-stabilized calcium phosphate nanoclusters.

Although the structural details of the casein micelle still remain elusive, it is generally accepted that κ-casein is located primarily, if not completely, on the micellar surface (Holt and Horne 1996). The hydrophilic region of κ-casein protrudes into the surrounding serum to give a "hairy" layer (5 to 10 nm thick) that provides steric and electrostatic stabilization (de Kruif and Zhulina 1996). Removal of the hairy layer by rennet or its collapse by acidification causes destabilization of the casein micelle (de Kruif 1999, Holt and Horne 1996).

The casein micelles are largely responsible for many of the technologically important physicochemical properties of traditional and new dairy products. Dried, frozen, and heated products, for example, rely on the ability of the casein micelles to retain their structural identity during concentration (e.g., ultracentrifugation, evaporation), freezing, homogenization, heat, and most other standard dairy processing operations (Fox and Kelly 2004). However, the casein micelle is also conducive to controlled destabilization routes (e.g., high pressure, acidification to pH 4.6, hydrolysis of κ-casein, alcohol, anionic detergents) (Fox and Kelly 2004), which are exploited in the manufacture of cheese and fermented dairy products.

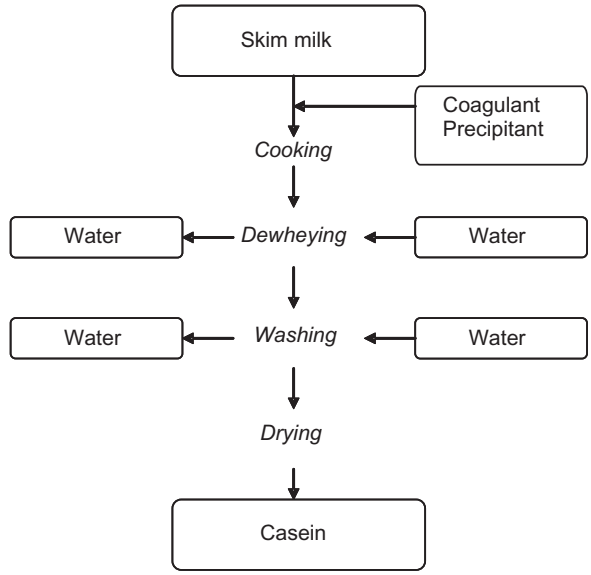

Figure 7.1. Process for manufacture of acid casein and rennet casein (Adapted from Huffman and Harper 1999).

Casein Products

Types of Casein Products

Due to its ease of production and natural abundance, casein has been produced commercially since the early 20th century. Hence, the technology for the production of casein products is well established and has been regularly improved (Mulvihill 1992, Mulvihill and Fox 1994, Mulvihill and Ennis 2003).

Two main types of casein are produced commercially: rennet casein and acid casein. Rennet casein is prepared by enzyme-induced coagulation. Acid casein is prepared by isoelectric precipitation using either mineral acid, an acidogen (e.g., gluconic acid-δ-lactone), or *in situ* fermentation of lactose to lactic acid using suitable lactic acid starter cultures. Other types of casein products are micellar casein, individual caseins, and casein-derived peptides.

Casein—Manufacture and Composition

Casein Manufacture

The principal steps involved in the manufacture of rennet casein and acid casein are outlined in Figure 7.1 and described in further detail below.

Fat Separation

Whole bovine milk is centrifuged to separate the cream (i.e., fat) from the skim milk that is then pasteurized (72°C; 161.6°F for 15 seconds) to eliminate pathogenic bacteria and inactivate indigenous enzymes.

Precipitation/Coagulation

Acid precipitation: Acid casein is precipitated from pasteurized skim milk by brief but turbulent agitation of the skim milk with dilute (0.25 mol L^{-1}) acid (sulphuric or hydro-

chloric) at 20°C (68°F), to pH 4.6 (Southward 1998). The casein precipitates as fine, discrete particles in a whey medium. An alternative acid for precipitation is lactic acid; the pasteurized skim milk is cooled to approximately 22°C to 26°C (71.6°F to 78.8°F) and then inoculated with lactic acid starter cultures (e.g., *Lactococcus lactis cremoris*) at 0.1% to 0.2% milk volume and incubated without agitation for 14 to 16 hours (Southward 1998). During incubation, the starter cultures ferment some of the lactose in milk to lactic acid and the pH decrease (pH approximately 4.6) causes coagulation of the milk. A soft casein gel (i.e., coagulum) is produced.

Rennet-induced coagulation: In the manufacture of rennet casein, the caseins are coagulated through the action of rennet. In contrast to the process used to prepare acid casein there is no change in the pH of the milk prepared by enzyme coagulation. Chymosin, the principal protease in bovine rennet, or various other types of proteases (e.g., microbial rennets, fermentation-produced chymosin) coagulate milk in a two-stage process. The primary stage involves the rennet-specific hydrolysis of the Phe_{105}-Met_{106} peptide bond of κ-casein to release the casein macropeptide (CMP) into the surrounding whey (Southward 1998). This results in a decrease in zeta-potential and loss of casein micelle steric stabilization. The remaining (N-terminal) κ-casein, referred to as *para*-κ-casein, is unable to protect the casein-sensitive caseins, and the caseins coagulate (i.e., clot) in the presence of calcium ions (Southward 1998).

When approximately 80% of the κ-casein has been hydrolyzed, the second stage of coagulation occurs (Fox and Kelly 2004). During this stage, under quiescent conditions, and providing a critical concentration of calcium is present and the temperature is above 18°C (64.4°F), the CMP-depleted micelles aggregate into chains or clumps, eventually forming a viscoelastic, three-dimensional gel (Ennis and Mulvihill 1999). The rennet coagulum consists of casein, whey protein, fat, lactose, and the minerals of the milk, and has a fluffier and spongier texture than acid precipitated casein.

Wet Processing of Casein Curd

Once the casein has been precipitated/coagulated it is broken up and then heated (60°C to 75°C; 140°F to 167°F for up to 30 minutes) (Ennis and Mulvihill 1999). Heating the precipitated casein expels excess moisture and causes the particles to shrink and agglomerate together to form clumps of curd. The curd is separated from the whey and washed several times in water (30°C to 40°C; 86°F to 104°F) (Ennis and Mulvihill 1999). The curd is then mechanically pressed or centrifuged to approximately 55% moisture content (Ennis and Mulvihill 1999).

Drying and Dry-processing Operations

The pressed casein curd is dried by hot air (approximately 130°C; 266°F) (e.g., using fluidized beds) to approximately 10% to 12% moisture content (Ennis and Mulvihill 1999). The warm, "dry" casein is then subjected to a series of dry-processing steps that involve cooling, tempering (to ensure the homogenous distribution of moisture), milling, sifting (to produce coarse, medium, and fine casein particles), and blending (to ensure homogeneity) (Ennis and Mulvihill 1999, Southward 1998). The casein is then bagged and either placed in storage or shipped immediately.

The yield of commercial casein using these processes is typically approximately 3 kg/100 kg skim milk (Southward 1998). Most of the acid casein is converted to a water-soluble salt form (i.e., caseinate). The rennet casein that is produced is used on an as-is basis.

Table 7.2. Typical composition of casein products. Southward (1998).

% Component	Rennet casein	Acid casein
Protein	79.9	85.4
Moisture	11.4	11.4
Ash	7.8	1.8
Fat	0.8	1.3
Lactose	0.1	0.1
Calcium	2.6–3.0	0.1
Phosphorus	1.4	0.6
Sodium	<0.1	<0.1
pH	7.3–7.7	4.6–5.4

Typical Compositions of Commercial Casein Products

The typical composition of rennet casein and acid casein are provided in Table 7.2. The major difference between rennet casein and acid casein is their mineral (ash/calcium) content and the pH of a water extract. During the acidification process in the manufacture of acid casein, the calcium and inorganic phosphate associated with the casein micelles in milk are dissolved and leached from the curd, leaving only a small residue of calcium and the organic phosphate. Rennet casein contains approximately 3% calcium and 1.4% phosphorous (Southward 1998). Native micellar casein is reported to have a similar composition to that of calcium caseinate, with 90% total protein (Fox and Kelly 2004).

Micellar Casein

Micellar casein, also called phosphocasein, is produced from milk by microfiltration performed on membranes with an average pore size of 0.1 to 0.2 µm (Fox and Kelly 2004). Microfiltration results in the removal of the whey proteins, lactose, and soluble salts with retention of the micellar casein and its associated CCP. This is in contrast to when casein is separated from the whey proteins by isoelectric precipitation of the casein or rennet-induced coagulation , which disrupt the natural micellar form of casein during manufacture.

There is no pH adjustment involved in the process. The fresh, liquid micellar casein concentrate has three-fold the protein content of skim milk (Barbano 2004). The micellar casein can be readily spray dried into a powder form. Casein can also be re-micellized using acid casein with pH adjustment to 7 and concomitant re-introduction of CCP (Mounsey et al. 2005b). Re-micellized casein has intrinsically higher ionic strength than native micellar casein, which impacts its functional properties (Mounsey et al. 2005b).

Caseinates

Types of Caseinate Products

Caseinates are the water-soluble forms of the casein, obtained by addition of alkali to freshly precipitated casein or dry casein powder. Various types of alkali may be used. When sodium hydroxide is used as the solubilizing alkali to neutralize the casein slurry, the resulting product is called sodium caseinate. Similarly, potassium, calcium, or ammonium caseinate are produced when potassium, calcium, or ammonium hydroxide, respectively, are used as the neutralizing agent. The caseinate solution is then dried using either a spray or roller dryer to produce a powder with a moisture content ranging between 3% and 8% (Southward 1985).

Sodium caseinate, ammonium caseinate, and potassium caseinate have similar physical properties. Their aqueous solutions are translucent, straw-colored, and viscous. In contrast, solutions of calcium caseinate are turbid (milky) because calcium caseinate forms aggregates in water. The increase in turbidity and decreased viscosity is due to the change in conformational and aggregation mode of the casein molecules in the presence of the calcium ions (Alvarez et al. 2008).

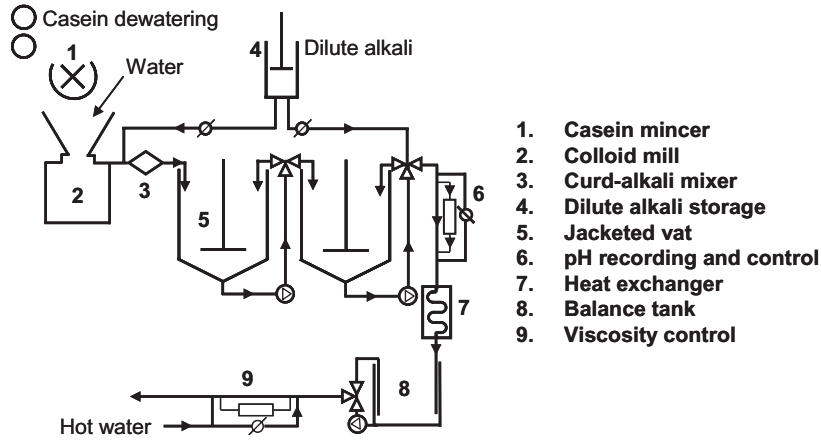

Figure 7.2. Proposed plant for manufacture of sodium caseinate. Adapted from Southward (1985).

Caseinate—Manufacture and Composition

The manufacture of caseinate has been previously reviewed (Mulvihill 1992, Southward 1985). A plant layout for the manufacture of sodium caseinate can be found in Figure 7.2.

Fresh curd from the dewatering press is minced to break up lumps and then slurried in water at 23% to 25% total solids. This slurry is passed through a colloid mill to reduce the curd particle size and produce a fine dispersion of casein curd particles in water. The reduction in particle size, prior to addition of alkali, is believed to reduce the time for dissolution of the curd. The addition of dilute alkali is carefully controlled to achieve neutral pH.

The dissolving operation is performed in the caseinating vats. These are high shear vessels with high-speed pumping facilities for recirculation, to ensure good mixing of the viscous caseinate solution prior to drying. The number of dissolving vats, typically three to five in a series, varies depending on the throughput of the dryer. To reduce the viscosity of the caseinate solution, jacketed vats are used to quickly raise the temperature of the casein with added alkali solution to 65°C to 75°C (149°F to 167°F). However, care should be taken to avoid holding the solution at above 70°C (158°F) for extended periods, because this can result in the development of brown color. Care should be also taken to keep the incorporation of air to a minimum, because sodium caseinate solutions form highly stable foams (Towler 1976).

After a total time in the caseinating reactors, usually 30 to 60 minutes, the caseinate solution is pumped through a final tubular heater (90°C to 95°C; 194°F to 203°F) to reduce its viscosity prior to spray draying. The sodium caseinate solution formed has a very high viscosity and is normally atomized into the spray dryer at 130°C (266°F) and at low total solids (20% total solids). Drying from low total solids (20% total solids) incurs high costs. Hence, a number of approaches have been used in an effort to reduce these costs (Muller 1983), including use of roller drying and manufacture of extruded and granular caseinates. It should be noted that commercial caseinate manufacturing plants can differ. For example, alkali can be added at different points in different plants.

Caseinates have similar composition, which is close to that of dry acid casein. However, they have a lower moisture content (typically about 4%) and a higher ash content

Table 7.3. Typical composition of caseinates. (Huffman and Harper 1999).

% Component	Sodium caseinate	Calcium caseinate
Protein	90.4	90.5
Moisture	4.6	4.6
Ash	3.8	3.7
Fat	1.1	1.1
Lactose	0.1	0.1
Calcium	0.03	1.3
Sodium	1.2	0.01
pH	6.8	6.8

(typically 3.7%) because of the alkali used in dissolution (Table 7.3). Sodium caseinate contains 1.2% to 1.4% sodium, whereas calcium caseinate contains 1.3% to 1.6% calcium. Caseinates have a pH in the range of 6.5 to 7.0.

Milk Protein Concentrates

Milk protein concentrates are concentrated forms of milk proteins that contain both the caseins and the whey proteins. The traditional method for producing milk protein concentrates was to co-precipitate the casein and whey proteins by heating skim milk, precipitating the complex between denatured whey protein and casein with acid/calcium chloride, and drying to obtain a powder. By varying the processing conditions, milk protein co-precipitates (80% to 90% protein) with different amounts of calcium and functional properties can be obtained (Vattula et al. 1979). This traditional method has been now been replaced by ultrafiltration for concentration of milk in the production of milk protein concentrates and isolates. Unlike the traditional process for total milk protein co-precipitates, in which the structure of the milk proteins is destroyed, concentration by ultrafiltration does not significantly change the milk protein structure.

During ultrafiltration, water, lactose, and mineral salts are removed in the permeate stream and protein is concentrated (Peri et al. 1973, Thompson and deMan 1975). The retentate is enriched in protein but depleted in lactose and soluble milk salts, and the milk minerals associated with the casein micelle remain. By using ultrafiltration alone, milk protein concentrates of up to 65% protein can be obtained. For production of milk protein concentrate powder with more than 65% protein, diafiltration is required. By adding water in the filtration process, the lactose and soluble milk salts can be further washed out, leading to an increased protein content of the retentate and therefore a higher protein powder (Getler et al. 1997).

Milk Protein Concentrates—Manufacture and Composition

Manufacture of Milk Protein Concentrates

The basic process for manufacture of skim milk concentrates is given in Figure 7.3. Typically, skim milk is pasteurized and ultrafiltered/diafiltered at the normal pH of milk. This process removes water and dissolved solids (i.e., lactose and mineral salts that are soluble in the serum phase of milk). The ultrafiltered retentate contains the total milk protein with the same ratio of casein : whey protein. The caseins in the retentate that are isolated using this process are in micellar form, their natural state in milk.

Skim milk is typically concentrated five- to six-fold, which concentrates the protein from 3.5% in skim milk to approximately 19% in the retentate, resulting in a powder with 70% to 80% protein on drying (Zwijgers 1992). The protein content of the retentate is further increased by diafiltration for production of milk protein isolate powders with higher protein content (approximately 90% protein in dry matter).

The ultrafiltration can be carried out using a cold (e.g., 10°C; 50°F) or hot (e.g., 50°C;

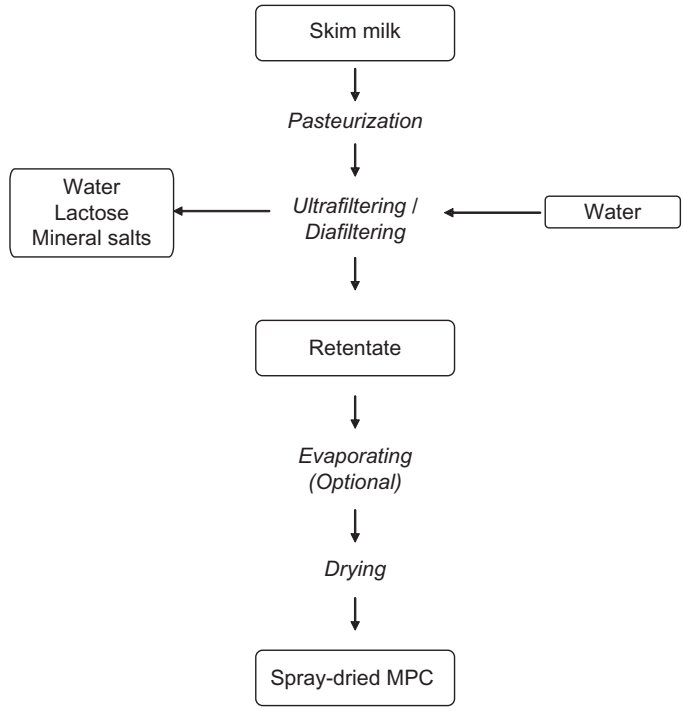

Figure 7.3. Process for manufacture of milk protein concentrate (MPC) powder.

122°F) process. The level of concentration of the retentate obtained depends on whether the cold or hot process is used and the type of membrane or combination of membranes employed (i.e., spiral wound, plate and frame). Using the cold process, milks can be concentrated to 20% to 24% total solids. More of the water is taken out in an evaporator (normally a falling film tubular evaporator) to obtain a higher total solids concentrate, typically with 32% to 35% total solids, prior to spray drying. When the hot process is used, concentrates of 28% to 33% total solids can be achieved and the evaporation step may be omitted (Getler et al. 1997).

Various spray dryers may be used for drying. The operating conditions of the dryer, such as the inlet and outlet temperatures, affect the characteristics of the milk protein concentrate powders. Increasing the inlet or outlet temperatures of the dryer can be detrimental to the powder properties. For example, increasing the outlet temperature of a pilot-scale spray dryer equipped with a two-fluid nozzle from 65°C to 90°C (149°F to 194°F) decreased solubility (pH 7.0) and rate of rehydration of milk protein concentrates with 70% protein (MPC 70) (de Castro and Harper 2001). Drying milk protein concentrates (MPC 70) using higher inlet temperatures (i.e., increasing temperature from 200°C to 250°C and 300°C; 392°F to 482°F and 572°F) in a pilot-scale spray dryer reduced the rate of rehydration of the powder (de Castro and Harper 2003).

For commercial operations, a tall form dryer coupled to an external fluidized bed for cooling the powder is desirable to obtain a high-quality milk protein concentrate powder (Getler et al. 1997).

Table 7.4. Typical composition of milk protein concentrate powders with 75% and 80% crude protein (MPC 75 and MPC 80) compared to skim milk powder. Adapted from Anon (1991), Zwijgers (1992).

% Component	Skim milk powder	MPC 75	MPC 80
Protein	36	75	80
Moisture	4.0	5.5	5.0
Ash	7.7	7.5	6.5
Fat	1.0	1.5	2.0
Lactose	51.3	19.5	6.5

Typical Compositions of Milk Protein Concentrates

Typical compositions of the major components of milk protein concentrate powders are given in Table 7.4. The content of calcium and phosphorus is higher in the milk protein concentrate than in skim milk (dry matter basis). For example, skim milk powder contains 12,500 ppm Ca and 10,000 ppm P, whereas milk protein concentrates with 80% crude protein (MPC 80) contain 23,300 ppm Ca and 14,500 ppm P. However, the contents of the monovalent ions in skim milk powder (4,800 ppm Na, 16,500 ppm K and 11,000 ppm chloride) are higher than those in MPC80 (1,100 ppm Na, 2,400 ppm K and 500 ppm chloride) (Zwijgers 1992).

There can be variations in the mineral content of milk protein concentrate powders with the same content of protein. This is not only because of compositional differences in the skim milk, but because differences in mineral composition also depend on whether a cold (e.g., 10°C; 50°F) or hot (e.g., 50°C; 122°F) ultrafiltration process is employed during milk protein concentrate manufacture. Due to the increased solubility of the calcium phosphate at low temperature, the retentate obtained by cold ultrafiltration is more depleted in milk minerals than that obtained by hot filtration. Other process modifications can also lead to differences in the composition of the milk protein concentrate powders, such as the addition of monovalent salts to ultrafiltered concentrate to improve the functional properties of the powder (Carr et al. 2004).

Functional Properties and Applications

The nutritional and functional properties of casein-based products make them useful ingredients for a range of products. Milk proteins have always been highly regarded for their high nutritional value. The caseins and caseinates, as well as the milk protein concentrates, have very good amino acid profiles, and the milk protein concentrates have the advantage of being high in bound calcium (Zwijgers 1992). The caseins, caseinates, and milk protein concentrates also have desirable flavor properties—from the relatively bland caseins to the milky flavor of the milk protein concentrates, which can have benefits in different applications.

Casein-based products are primarily used as food ingredients to modify the physical properties (e.g., water-binding, viscosity) of the food or to increase its nutritive value. Consequently, they are often a minor ingredient in food. However, the type of foods in which casein-based products can be used is wide-ranging and includes applications in vitamin and mineral tablets, instant drinks, various nutrition foods (dietetic foods, nutritional food bars, sports drinks), pasta, infant foods, some cereal products, sauces, gravies, soups, stews, baked products (e.g., bread, cake products), sweet goods, pastries, cus-

tards, aquatic feeds, ice cream, spreads, fillings and creams (e.g., synthetic cream whip), cream liquers, puddings, and fabricated meats (Fox and Kelly 2004).

The functional properties of casein-based ingredients are affected by various factors, including changes in pH, method of preparation, ionic strength, and the nature of the salt ion present in the preparation. Some of the key physical properties of casein-based powders are discussed below. It is the physical functional properties that often dictate their suitability for an application. Specific examples of their physical properties that relate to their functionality as food ingredients are described.

Solubility/Hydration

The solubility and hydration of protein ingredients is a pre-requisite for other physical functional properties of milk proteins, with insolubility or inadequate hydration compromising functionality in many applications.

Casein: The hydration of rennet casein relies on disruption of the calcium-mediated protein-protein interactions to increase the protein-aqueous solvent interactions (Aimutis 1995, Caric and Kalab 1993, Ennis and Mulvihill 1997). The pH of the solution, and concentration and type of sequestering salt used to disperse the casein, influences the extent of its hydration (Ennis et al. 2000, Savello et al. 1989). Differences in protein and mineral (ash and calcium) contents, as a result of different heat conditions in the pasteurization of skim milk prior to rennet manufacture (Ennis and Mulvihill 1999, O'Sullivan and Mulvihill 2001) and heat treatment during rennet casein manufacture, may also affect the hydration of rennet casein (O'Sullivan et al. 2002a, 2002b).

The extent and nature of casein hydration are critical factors in determining its functional performance. An example of the effect of hydration on functionality is the use of rennet casein during the manufacture of cheese analogues (O'Sullivan and Mulvihill 2001). Insufficient hydration of rennet casein can cause under-emulsification of the oil phase (i.e., large oil droplets are formed) during manufacture of the cheese analogue (Aimutis 1995, Ennis et al. 2000). Consequently, the cheese analogue that is produced may exhibit poor stretchability and free oil release on heating. If excessive solubilization of the casein occurs, then over-creaming or over-emulsification (i.e., very small oil droplets are formed) can occur during manufacture of the cheese analogue (Meyer 1973). In this case, the cheese analogue exhibits poor meltability and decreased flow characteristics on heating (Savello et al. 1989), which are undesirable in mozzarella cheese analogues intended for pizza toppings, for example.

Micellar casein: Rehydration of spray-dried micellar casein powders prepared by membrane microfiltration is slow when compared, for example, to low-heat milk powder (Schuck et al. 2007). This has been attributed to low water transfer into the casein micelle (Schuck et al. 2007). Rehydration of the powder can be improved by adding citrate or phosphate to the suspension prior to drying or addition of NaCl during the rehydration process (Schuck et al. 2002). In contrast, addition of $CaCl_2$ has an adverse effect, producing insoluble structures during spray drying (Schuck et al. 2002).

Water absorption capacity, water solubility, and water-holding capacity are higher for micellar casein than for rennet casein (Roman and Sgarbieri 2006).

Caseinate: Although caseinates are credited with very good solubility, this strongly depends on different conditions such as pH, ionic strength, and temperature. For instance, at pH from 5.6 to 6.2, the solubility of both sodium caseinate and calcium caseinate is improved when sodium phosphate is added (Konstance and Strange 1991). Sodium caseinate has the lowest solubility near its pI (i.e., pH around 4.6) and the solubility of

sodium caseinate increases dramatically as the pH is increased from 6.5 to 8.0 (Jahaniaval et al. 2000). Bastier et al. (1993) compared the solubility of several commercial caseinates at pH 7.0. They reported that solubility of calcium caseinate was not significantly different from that of sodium caseinate, but was significantly lower than that of potassium caseinate.

Milk protein concentrates: The solubility of milk protein concentrate powders (MPC powders) is low compared to that of skim milk powders. The solubility of the powders deteriorates on storage, particularly at elevated temperatures. A survey of commercially available MPC powders with 82% to 86% protein showed that there was wide variability in the solubility of these powders, although the cause of the variation was not investigated in that study (de Castro-Morel and Harper 2002).

The nature of the insoluble material has been examined. McKenna (2000) found that the insoluble material obtained on dispersion of MPC85 that had been stored for six months at 20°C (68°F) was mainly fused casein micelles. Havea (2006) demonstrated that the material was primarily α- and β-caseins that were associated by weak non-covalent protein-protein interactions.

Studies on the rehydration of MPC 85 suggest two processes control the dissolution of the powders, namely the disruption of agglomerated particles into primary powder particles and the release of material from the particle into water (Mimouni et al. 2009). The addition of monovalent salts is claimed to improve the solubility of the milk protein concentrate and milk protein isolate powders (Carr et al. 2002). However, simply increasing the temperature of the water increases the dissolution rate of milk protein concentrate powders. MPC powders should be dispersed in water at temperatures of 40°C to 60°C (104°F to 140°F) with a high energy dissolution unit (e.g., Venturi) for optimum solubility (Zwijgers 1992).

Viscosity and Gelation

The ability of proteins to build viscosity and to gel enables them to be used in a variety of applications. Functional tests that examine the properties of the proteins are sometimes used to screen protein-based ingredients for specific applications (Ennis and Mulvihill 1999).

Casein: The most important application of casein-based products is in cheese making and cheese analogues (processed cheese), in which the casein's ability to form gel networks is used. Rennet casein is the primary protein source used in mozzarella cheese analogues due to its desired sensory attributes (pale color, bland flavor) and superior functionality (firmness, stretchability, shreddability, emulsification and melt) over alternate protein sources (Aimutis 1995, Ennis and Mulvihill 1997).

Acid casein has been shown to display useful properties in baked goods. For example, an aggregated acid casein-based ingredient fortified with calcium has the potential to replace the highly functional covalent (disulfide) bonds in wheat dough to produce gluten-free breads and cakes (Stathopoulos and O'Kennedy 2008). The aggregated casein samples (30 mg Ca/g sample) were more elastic than gluten, but their behavior on heating above 40°C (104°F) produced materials that were weaker and more viscous. Hence, further improvements are required to improve its heat stability.

Micellar casein: Micellar casein displays excellent rennet-coagulation properties. A 3% miceller casein suspension reduced coagulation time by 53% compared to that of raw milk, and increased gel firmness at 30 minutes by more than 50% (Pierre et al. 1992). Remicellized casein produced stronger rennet gels but had poorer acid gelation properties than native micellar casein (Mounsey et al. 2005b).

Micellar casein is particularly well suited for cheese making (Saboya and Maubois

2000). It increases the protein content of cheese milk, thereby improving the quality of cheese and increasing the capacity of a cheese plant (Kelly et al. 2000). Garem et al. (2000) described the production of a micellar casein milk powder using a combination of microfiltration and ultrafiltration with improved cheese yielding capacity. Mozzarella cheese yield was 7.3% higher in comparison to control cheese prepared from fresh milk. A three-stage microfiltration process that removes 95% of the whey proteins from skim milk prior to cheese making has also been developed with the potential for continuous production of cottage cheese without acid whey production (Nelson and Barbano 2005).

Caseinate: As a consequence of their hydration and protein-protein interactions, solutions of caseinates exhibit very high viscosity. The viscosity increases rapidly (usually exponentially for sodium caseinate) with concentration. However, the viscosity of calcium caseinate is much lower than that of sodium caseinate as it exists in water as a colloidal dispersion (Southward 1985). Caseinate solutions exhibit Newtonian behavior at low protein concentrations and a pseudoplastic behavior at high concentrations, and are thixotropic at high shear rates.

The viscosity of caseinate solutions also increases with salt concentration and decreases with temperature. Carr et al. (2002) reported that the addition of monovalent cations (K^+, Na^+, and NH_4^+) exponentially increased the viscosity of sodium caseinate solutions due to the competition of the salt for water, resulting in an effective increase in protein concentration. However, divalent cations (Ca^+, Mg^+, and Zn^+) increased the viscosity of sodium caseinate solution to a maximum, and then decreased it, due to protein aggregation. The viscosity of sodium caseinate also strongly depends on pH, with a minimum at pH 7.0 and higher viscosity at low pH (2.5 to 3.5) than at neutral pH (Mulvihill 1992). The viscosity is not markedly affected by the pH in the range of 6.5 to 7.0, but reaches a maximum value at about pH 9.5 (Carr et al. 2002).

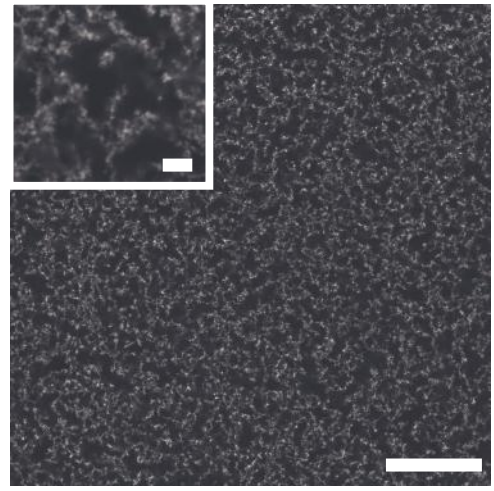

Figure 7.4. Confocal micrograph of 5% sodium caseinate gel induced by the addition of 2% glucono-δ-lactone at 30°C for 3 hours. Bar scale represents 50 μm; inset 5 μm.

Solutions of caseinate form gels on acidification (Braga et al. 2006) due to the formation of a protein network. An example of the microstructure of acid-induced gelation of sodium caseinate is shown in Figure 7.4, where a protein network (appears in white) is formed. Recently, reversible cold gelation, induced by salt addition and refrigeration, of sodium caseinate solution was also reported (Carr and Munro 2004). Gelation can also be achieved, for example, via cross linking the protein by addition of transglutaminase (Dickinson and Yamamoto 1996).

Milk protein concentrates: Milk protein concentrate dispersions have lower viscosity than sodium caseinate and calcium caseinate dispersions at an equivalent protein concentration (Zwijgers 1992). This is related in part to caseins having a higher capacity to bind water than whey proteins. Micellar casein holds approximately 3.3 g water/g casein and undenatured whey protein holds approximately 0.4 g water/g protein (Walstra and Jenness 1984).

Milk protein concentrates have applications in cheese making as the micellar casein participates in gel network formation. When milk protein concentrates with high protein content (e.g., MPC 70 to 85) are used, added calcium is required to form rennet gels (Kuo and Harper 2003). A major use of milk protein concentrates is in the standardization of cheese milk as an alternative to skim milk powder. One of the advantages of using milk protein concentrate powders for standardization of cheese milk is that it has lower lactose content than skim milk powder, resulting in cheese with reduced residual lactose. Modification of the cheese making procedure may be required to enhance flavor development and ripening of the cheese (Rehman et al. 2003).

Surface-Active Properties

The open and flexible structure of the caseins, combined with their amphiphilicity, imparts excellent surface-active properties (e.g., foaming, emulsifying) to the caseins (Fox and Kelly 2004). Euston and Hirst (2000) compared the emulsifying properties of commercial milk protein products. When the caseins in products were in a non-aggregated state (i.e., in sodium caseinate), they had higher emulsifying capacity than those in an aggregated/micellar state (i.e., in skim milk powder or milk protein concentrate).

The surface-active properties of acid precipitated and subsequently neutralized casein, rennet casein, and native micellar casein were compared, with the influences of pH and salt concentration taken into account (Roman and Sgarbieri 2006). Compared to micellar casein, emulsifying capacity was higher for acid casein at pH 4 and 7. Emulsion stability was high for acid casein at pH 4 and for micellar casein at pH 7. Foaming capacity was higher for micellar casein compared to acid casein at pH 4, but higher for acid casein at pH 6 and 8. Foam stability was low for micellar casein and acid casein at pH 4, but high for micellar casein at pH 6 and 8. Rennet casein displayed the lowest emulsifying capacity and did not foam under the conditions tested.

Sodium caseinates are extensively used as emulsifiers owing to the random structure and non-uniform distribution of hydrophobic and hydrophilic residues of the caseins. In emulsions, the protein load of sodium caseinate needed to stabilize oil-water interfaces is very low (1.4 to 3.7 mg/m^2), but depends on the physico-chemical conditions such as salt and sodium caseinate concentration (Srinivasan et al. 1996). However, although sodium caseinate is considered to be a good emulsifier, an excess in an emulsion formulation can lead to destabilization by depletion flocculation (Dickinson 2006). Calcium caseinates are also known to be good emulsifiers, although due to the aggregated state of the proteins, their surface load is higher than that of sodium caseinate, particularly when high protein concentration is used (Srinivasan et al. 1999).

Heat Stability

Heat stability is the ability of milk to withstand coagulation at high temperatures. Because heat treatment is an essential unit operation, heat stability is a desirable property for milk protein ingredients used in many applications.

Micellar casein is reported to display greater heat stability than acid casein or rennet casein (Barbano 2004). Heat stability was further increased at pH less than or equal to 6.6 at low- and high-ionic strength using re-micellized casein (Mounsey et al. 2005a). Heat stability was also markedly improved at pH above 6.8 following incubation with transglutaminase (Mounsey et al. 2005a).

Caseinates are more stable to heat than milk. However, extensive heating of caseinate solution can result in polymerization of the caseins as well as degradation of the pro-

teins by heat-induced proteolysis (Guo et al. 1989).

Milk protein concentrates are very stable to heat treatment, as observed by a comparison of protein emulsions (5% protein:5% fat) stabilized by MPC 80, sodium caseinate, or calcium caseinate heated at 130°C (266°F) for 13 minutes. The emulsions stabilized by sodium caseinate or MPC 80 were stable, whereas those made with calcium caseinate were destabilized. In addition, the milk protein concentrates that had the highest protein load on the surface of emulsion droplets were most stable to creaming on storage (Zwijgers 1992).

The Future

Casein-based products have traditionally been used for their ability to contribute to the physical and sensory properties of foods. There are emerging opportunities to increase the use of casein-based products for nutrition and functional foods.

An attractive value-added application of micellar casein is its use in shelf-stable, high-quality nutrition beverages (Barbano 2004). Micellar casein provides the mouth feel of milk with approximately 2% fat without the calories. An example is a low-fat, lactose-free nutrition beverage with excellent flavor that is produced using micellar casein.

Milk proteins and casein-based products are finding applications as encapsulating matrices for the delivery of bioactive ingredients through foods. When used in combination, or conjugated, with carbohydrates, casein-based products have the potential for site-specific delivery of oils and oil-soluble bioactives to the gastrointestinal tract (Head et al. 2005).

The casein micelle is a natural nanocapsule. Hence, it may be harnessed to deliver various food ingredients including vitamins (Semo et al. 2007) and bioactives (Sahu et al. 2008). Cross linking of the casein micelles by transglutaminase produces nanogel particles, which also may serve as delivery vehicles in food systems (Huppertz and de Kruif 2008).

Individual caseins and casein-derived peptides are gaining increasing attention due to their potential role in the developing market of physiologically functional foods, or nutraceuticals. Physiologically, casein-derived peptides have been implicated in the nervous, cardiovascular, digestive, and immune systems (Hartmann and Meisel 2007, Silva and Malcata 2005). Casein phosphopeptides, CMP, and caseinomorphines are the best studied casein-derived bioactive peptides (Kilara and Panyam 2003, Silva and Malcata 2005). Many of the products are commercially available in limited quantities and have been used as dietary supplements in functional foods and as pharmaceutical preparations. However, the postulated health benefits and the molecular mechanisms underlying their bioactivities *in vivo* are equivocal.

The versatility of casein-based ingredients and the potential to further differentiate them by improved separation and process modification procedures ensures that casein-based ingredients will continue to have a place as valued ingredients in the food industry.

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Chapter 8

Whey-based Ingredients

Lee M. Huffman and Lilian de Barros Ferreira

Introduction

According to the Food and Drug Administration Code (Title 21 CFR Section 184.1979), whey is the liquid substance obtained by separating the coagulum from milk, cream, or skim milk in cheese making. Whey also may be obtained from curd formation by the direct acidification of milk (casein manufacture). Figure 8.1 shows a simplified flow diagram for the production of whey, lactose, and whey ingredients.

It is estimated that more than 90% of whey originates from cheese making and that less than 10% originates from casein production (Affertsholt and Nielsen 2007). During cheese manufacture, 10 kg of milk generates roughly 1 kg of cheese and 9 kg of whey. The whey stream contains approximately 6% solids, mainly lactose, minerals, whey proteins, fat, and byproducts of cheese (or casein) manufacture. The quality and composition of the whey stream vary, depending on the type of cheese (or casein) being produced and manufacturing practices (Patel et al. 1990, De la Fuente et al. 2002).

In 1959, only 27% of the total whey produced was used by the food industry (animal and human). Whey has a high chemical oxygen demand (COD; 73 to 86 kg/m^3) and a high biochemical oxygen demand (BOD; 38–46 kg/m^3); environmental concerns and regulations have reduced the amount of whey that can be disposed (Bullerman and Berry 1966, Farizoglu et al. 2004). By 2006 the industrial utilization of whey was around 80% in the United States and Canada, 60% in the European Union, 90% in Oceania, 40% in Brazil and Argentina, and 25% in the rest of the world.

Industrial use of whey is vital to the economic feasibility of the dairy industry in developed countries. In addition to the relevance of environmental concerns relating to whey disposal, the nutritional and functional properties of whey ingredients make whey a valuable product (Figure 8.2).

Technological advances in ultrafiltration since the early 1980s have allowed the development of the various whey products shown in Figure 8.1. The industrial use of whey began no more than 30 to 40 years ago, with ongoing research on product properties and creation of value-added whey ingredients of increasing relevance (Abd El-Salam et al. 2009). Recent technological advances in both membrane and chromatography technology have opened the way for further purification and fractionation of whey ingredients. The introduction of ceramic (Maubois and Ollivier 1991) and polymeric (Zulewska et al. 2009) microfiltration membranes has allowed the separation of whey proteins from casein proteins directly from milk, thus creating a new product of greater value.

In Figure 8.2 the size of the bubble is proportional to the commercial value of the whey or lactose ingredient; values vary

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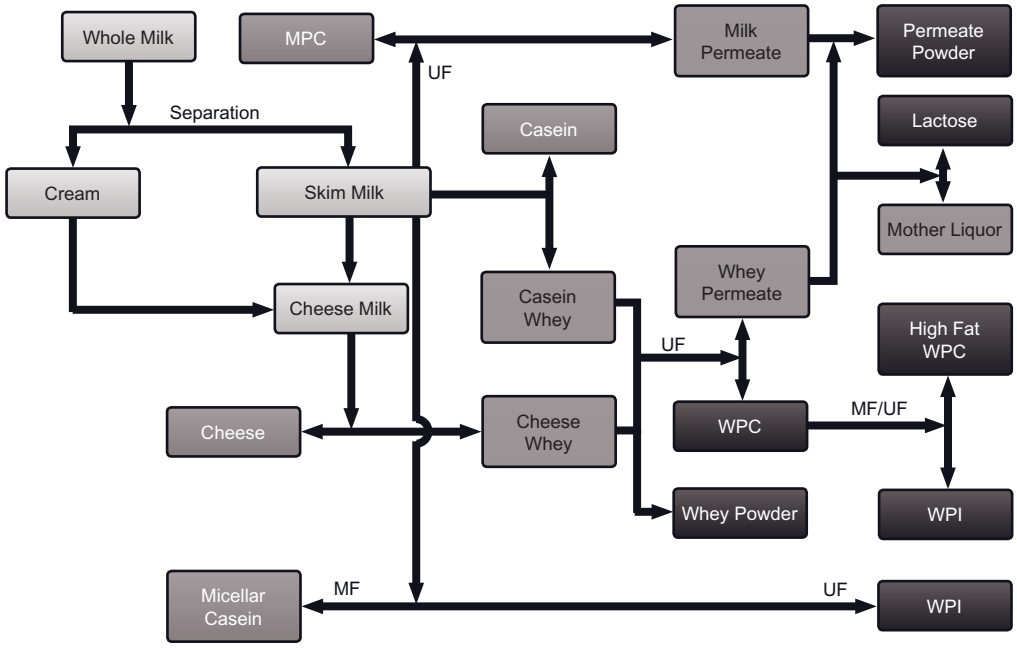

Figure 8.1. Flow diagram for the production of whey ingredients.

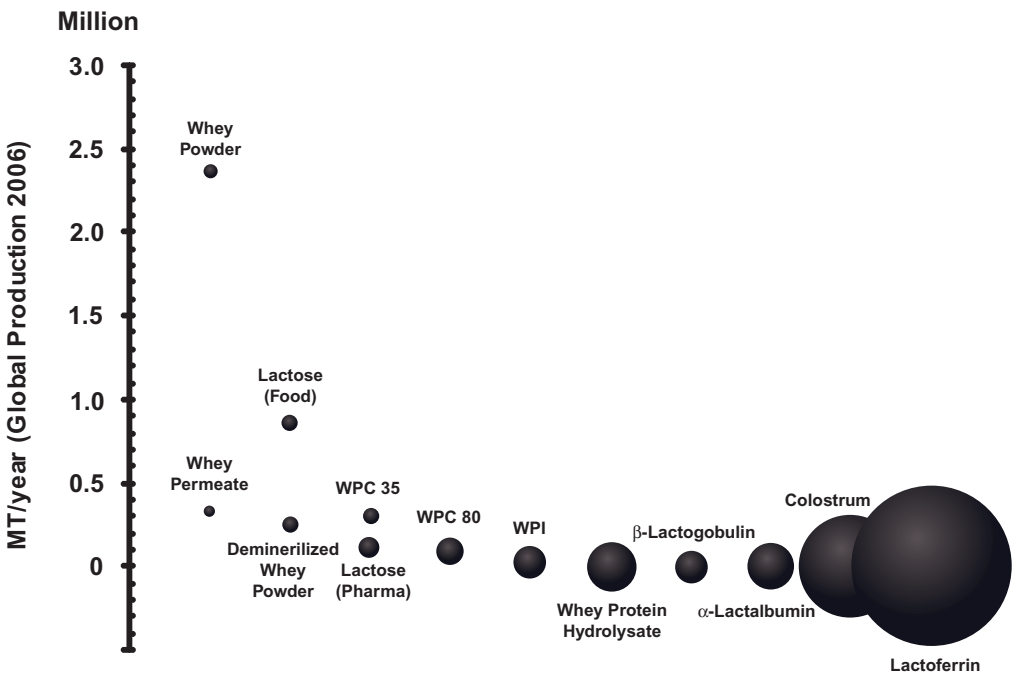

Figure 8.2. Volume of whey products produced globally in 2006. The size of the bubble is proportional to the commercial value of the product. Adapted from Affertsholt and Nielsen (2007).

between $1.50/kg and $280/kg. The placement of the bubble in relation to the Y axis is related to its total global production, as given by Affertsholt and Nielsen (2007).

Whey Sources and Composition

Whey is a dilute liquid containing approximately 6% solids, of which approximately 5.4% is lactose and 0.7% is protein. The whey protein is a complex mixture of β-lactoglobulin (β-lg; approximately 55%), α-lactalbumin (α-lac; approximately 24%), bovine serum albumin (BSA; approximately 5%), immunoglobulins (approximately 15%), and several other minor proteins (Swaisgood 1996). Whey also can include more than 20% glycomacropeptide (GMP) if the whey is from cheese manufacture (Regester and Smithers 1991).

Whey produced from cheese and rennet casein is known as sweet whey; whey produced from direct acidification (e.g., from cottage cheese or acid casein manufacture) is known as acid whey. Whey produced from the microfiltration of skim milk is yet to receive a standard of identity; it has been named serum protein, soluble milk protein, and native WPI (Marcelo and Rizvi 2008, Zulewska et al. 2009). The whey compositions are shown in Table 8.1.

Whey composition is variable and depends on a number of factors (Abd El-Salam et al. 2009):

- Use of rennet (chymosin) in cheese and rennet casein manufacture adds glycomacropeptide to the whey stream. This protein fraction is not present in whey streams derived from the direct acidification or the microfiltration of skim milk. GMP increases the protein content of the whey fraction and changes its functional properties by diluting the effects of β-lactoglobulin and α-lactalbumin.
- Cheese type impacts pH, total solids, and mineral and fat contents; cheese manufacture also adds cheese fines, various protein peptides, enzymes, and starters.
- Use of direct acidification leads to a whey stream with higher mineral content and adds acid ions that change the flavor and functional properties of the whey.
- In general, casein whey, which is made from skim milk, has lower fat content than cheese whey, made from whole milk. This difference in initial whey fat is reduced by separation of the fat from cheese whey during whey treatment.
- Whey protein from the microfiltration of skim milk typically has no measurable fat and may contain small amounts of casein proteins (Oestergaard, no date; Marcelo and Rizvi 2008).
- Seasonal variation in the composition of milk and whey affects dairy herds that are grass fed and receive minimal supplementary feeding. Calving occurs in spring and

Table 8.1. Typical composition of whey streams.

	Sweet whey	Acid whey	Soluble whey protein from microfiltration
% Total solids	6.4 ± 0.4	6.4 ± 0.4	5.8 ± 0.3
% Protein	0.85 ± 0.05	0.7 ± 0.2	0.55 ± 0.05
% Lactose	5.0 ± 0.1	4.6 ± 0.3	4.6 ± 0.2
% Fat	0.06[a]–0.4[b]	<0.05[a]	Not detected
% Ash	0.65 ± 0.05	0.75 ± 0.05	0.55 ± 0.05
% Calcium	0.04–0.05	0.15 ± 0.5	No data
pH	5.9 ± 0.2	4.5 ± 0.1	6.6 ± 0.1

[a]After separation
[b]Before separation
Adapted from Zadow (1992), Anon (2004), Kilara (2008)

herds usually produce milk for only 8 to 9 months during a typical milking season. Seasonal changes in the milk composition result from variation in the nutritional intake of the milking herd. During the last three months of lactation, the α-lactalbumin content of whey declines and the contents of β-lactoglobulin and glycomacropeptide contents are maximized (Regester and Smithers 1991).

Chemistry of Whey Proteins

The whey proteins found in whey streams include globular proteins (β-lactoglobulin, α-lactalbumin, and bovine serum albumin), immunoglobulins (Ig-G, Ig-A, and Ig-M), some minor proteins (lactoperoxidase, lactoferrin, and lysosyme), and polypeptides (proteose-peptone 3, and proteose-peptone 5) and glycomacropeptides (present in rennet casein or cheese whey only) (Swaisgood 1996). The functional properties of whey ingredients are defined by the physicochemical properties of the whey proteins that contribute to the desired characteristics in food products (Mangino 1984). The conformation and functional properties of whey proteins are interrelated and governed by changes in the globular folded structure of the molecule (de Wit 1989). In order to understand the behavior of whey proteins in food systems, knowledge of both the physicochemical properties of individual proteins and the effects of environmental factors (process history, product composition, thermal treatment, etc.) is required (Regester et al.1992, De la Fuente et al. 2002a). In addition to physico-chemical properties, various proteins present in whey have important biological properties (Harper 2000, 2001).

β-lactoglobulin comprises more than 50% of the whey proteins in bovine milk. Because of its prevalence in bovine milk, the properties of β-lactoglobulin determine the properties of whey protein concentrates (Abd El-Salam et al. 2009).

In the pH range 4 to 5, β-lactoglobulin is insoluble in aqueous solutions with low salt concentration. During extensive demineralization of whey, as achieved by ion exchange, conditions may lead to a loss of protein solubility (de Wit 1981). Protein solubility is relevant in the formulation of whey-containing beverages and is affected by pH, protein concentration, and concentrations of salts and other ingredients such as sugars. In general, sugars may protect proteins against the loss of solubility by heat treatment. The least heat-sensitive pH range of the whey proteins lies between 2.5 and 3.5. Whereas the susceptibility of whey proteins to denaturation is largely determined by the pH of the solution, the extent of protein aggregation depends on the presence of calcium salts (de Wit 1981).

Heating is universally applied during the industrial processing of foods. The increase in heat sensitivity of proteins in the presence of calcium is believed to be due to the formation of ionic intermolecular cross links that increase the proximity of the molecules. With α-lactalbumin, however, calcium is used in the formation of intramolecular ionic bonds that tend to make the molecule resistant to thermal unfolding (Kilara 2008).

β-Lactoglobulin contains two disulfide bonds and one sulfhydryl group. It is through thiol/disulfide interchanges that protein aggregation is initiated. It is also through heat-induced sulfhydryl and disulfide interactions of α-lactalbumin with β-lactoglobulin that the heat sensitivity of α-lactalbumin is increased (de Wit 1981).

β-Lactoglobulin is not present in human milk. Treatments to reduce the allergenicity of bovine milk generally involve the hydrolysis of β-lactoglobulin.

Processing Techniques

In the processing of whey streams one objective is to reduce the water content of the product stream to recover dairy solids and preserve them for further use in formulated

foods. Another objective is to fractionate and isolate components of higher value. In the dairy industry a falling film evaporator and a spray dryer are commonly used for bulk reduction and ingredient preservation. Fractionation and isolation of whey proteins is achieved either by membrane filtration, through size selective permeability, or by ion exchange, through charge attraction to a porous surface.

Ultrafiltration (UF)

Ultrafiltration is a pressure-driven, cross-flow filtration process that concentrates whey proteins through polymeric membranes. The feed stream flows tangentially to the membrane which acts as a barrier to the permeability of selected molecules. In ultrafiltration the membrane has a pore size between 0.001 and 0.05 µm; proteins and fats are retained, whereas water, salts, sugars, and peptides may permeate through the membrane (Figure 8.3). The process yields two streams: a retentate with the concentrated protein solution and a permeate that is rich in lactose and salts.

It is possible to produce a retentate of 22% to 25% solids before the viscosity of this stream impairs the separation process. At this point the protein content of the retentate is about 60% to 65% of the dry matter. In order to obtain a retentate with a higher percentage of protein (up to 85%), water is added to concentrate the protein. The pressures used in this process are relatively low: 2 to 6 bar. UF membranes are typically purchased by the nominal molecular cut-off provided by the suppliers. However, the actual retention for a membrane depends on the fouling layer on the membrane which is controlled by the operating conditions (temperature and pressure), feed stream (composition and processing history), and membrane design. A diagram of a spiral-wound UF membrane unit is shown in Figure 8.4. Figure 8.5 is a photograph of a spiral wound membrane processing unit.

Nanofiltration (NF)

The membrane used in nanofiltration allows the preferential permeation of monovalent salts (e.g., sodium, potassium, and chloride) and retains divalent salts (e.g., calcium) and lactose. The separation mechanism involves both diffusion through the membrane material (as in reverse osmosis) and convective pore flow (as in ultrafiltration). Furthermore, separation of complex mixtures

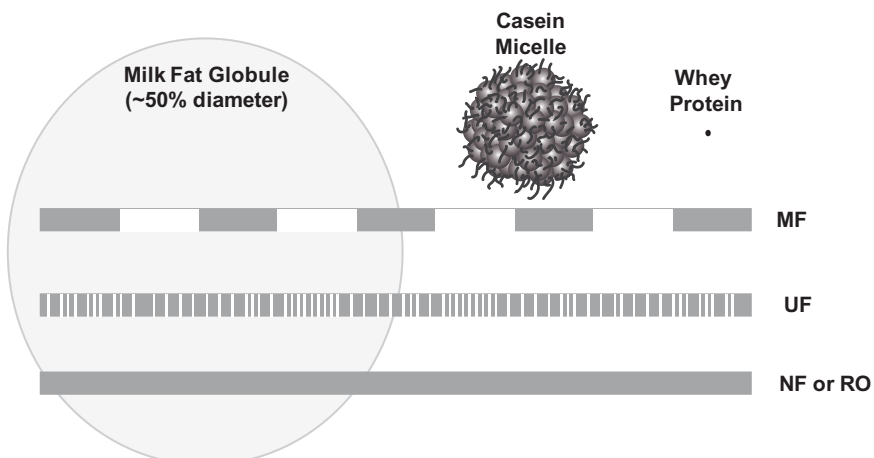

Figure 8.3. Relative size of whey components and membrane pore sizes. Reproduced by permission of Fonterra Co-operative Ltd.

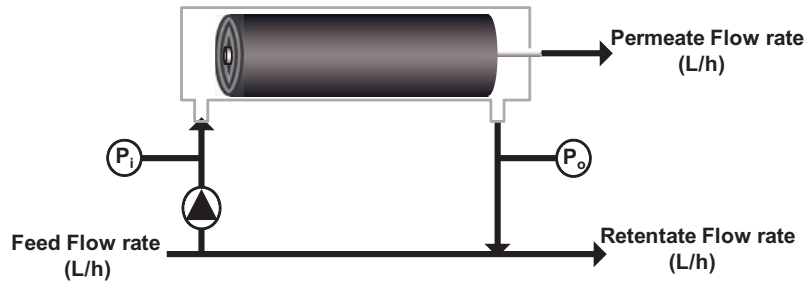

Figure 8.4. Spiral-wound UF membrane unit. Reproduced by permission of Fonterra Co-operative Ltd.

Figure 8.5. Spiral wound UF membrane unit. Reproduced by permission of Fonterra Co-operative Group Ltd.

is complicated by Donnan exclusion effects. Therefore, the rejection rate of an ionic species depends on not only membrane pore size, but also retentate concentration and the presence of various co-ions. The pressures used in this process vary between 8 and 20 bar.

Microfiltration (MF)

Microfiltration is used to separate large particles (fat, casein, microorganisms) from whey or milk. Residual fat must be fully separated from the protein to produce whey streams of 90% protein and greater.

In microfiltration the pore size of the membranes varies between 0.2 and 1.4 µm. For the defatting of whey streams, 0.1 to 0.2 µm membranes are commonly used (Figure 8.3).

The MF process was first introduced with tubular ceramic membranes, which have superior pore size uniformity, leading to good selectivity toward the permeability of whey proteins and the retention of fat. They also have a lifetime of more than 10 years.

However, a ceramic membrane system costs three to 10 times more than a spiral-wound, polymeric membrane system. That said, it is only recently that polymeric membranes capable of defatting whey with similar selectivity to ceramic membranes have been successfully developed.

The biggest issue in processing whey with microfiltration membranes is the balance between throughput—through greater permeability of the valuable components such as whey proteins—and membrane pore blocking. To control the membrane fouling and reduce the potential for membrane pore blocking, microfiltration is run at increased cross-flow velocities. The transmembrane pressure in ceramic membranes is kept uniform along the length of the module via co-current permeate flow or manufacture of membranes with a controlled gradient of resistance against permeation. The process operates under pressures below 1.5 bar.

Pre-treatment of the feed to microfiltration, for example, via heat treatment and pH adjustment, is also recommended to stabilize fat and protein aggregates (Merin and Daufin 1990), thus enhancing the separation ability of the process.

Ion Exchange (Protein Isolation and Fractionation)

Ion exchange chromatography is used to produce high purity whey proteins. The separation is based on the electric charge of the proteins, which is a function of the number and nature of the ionizable groups on the polypeptide chains. The separation by ion exchange chromatography is based on the fact that at a pH lower than the isolectric point (Table 8.2), proteins have a net positive charge and can be adsorbed on cationic ion exchangers, whereas, at a pH higher than the isoelectric point, they have a negative charge and can be adsorbed on anionic ion exchangers (Kaczmarek 1980).

In choosing conditions for adsorption, it should be noted that the tertiary structure of the whey proteins is altered by the effects of high and low pHs. These changes include unfolding, which is usually reversible, and the interaction of sulfhydryl groups and disulfide bonds, which is irreversible. Both can affect the efficiency of protein recovery because the unfolded or denatured protein can bind covalently and irreversibly to the ion exchanger. This can cause a decrease in the processing efficiency. Therefore, extreme pHs should be avoided (Howell et al. 1990).

Table 8.2. Isoelectric point of whey proteins.

Protein	Isoelectric point (pH)
β-Lactoglobulin	5.2–5.5
α-Lactalbumin	4.2–4.8
Bovine serum albumin	4.7–5.1
Immunoglobulins	5.5–8.3
Proteose-peptones	3.3–3.7

Adapted from Swaisgood (1996), Kilara (2008)

The separation of proteins by ion exchange chromatography occurs in four steps (Figure 8.6):

1. Adsorption. The whey stream is pH adjusted, diluted, and mixed or passed through the tank filled with ion exchanger. Only the proteins charged appropriately are adsorbed.
2. Washing. The tank is washed with enough water to displace any non-adsorbed components of the whey solution still in the tank.
3. Elution. A salt, acid, or basic solution is used to displace the proteins and concomitantly regenerate the ion exchanger.
4. Washing. The tank is washed with enough water to remove all the protein solution.

After the ion exchange treatment, the protein solution is often very dilute (3% to 5%) and contains a large quantity of salts. Ultrafiltration is recommended before evaporation and drying, both to increase the concentration of the stream and to reduce the amount of minerals.

Etzel (1995) reviewed the existing methods for isolating proteins from whey

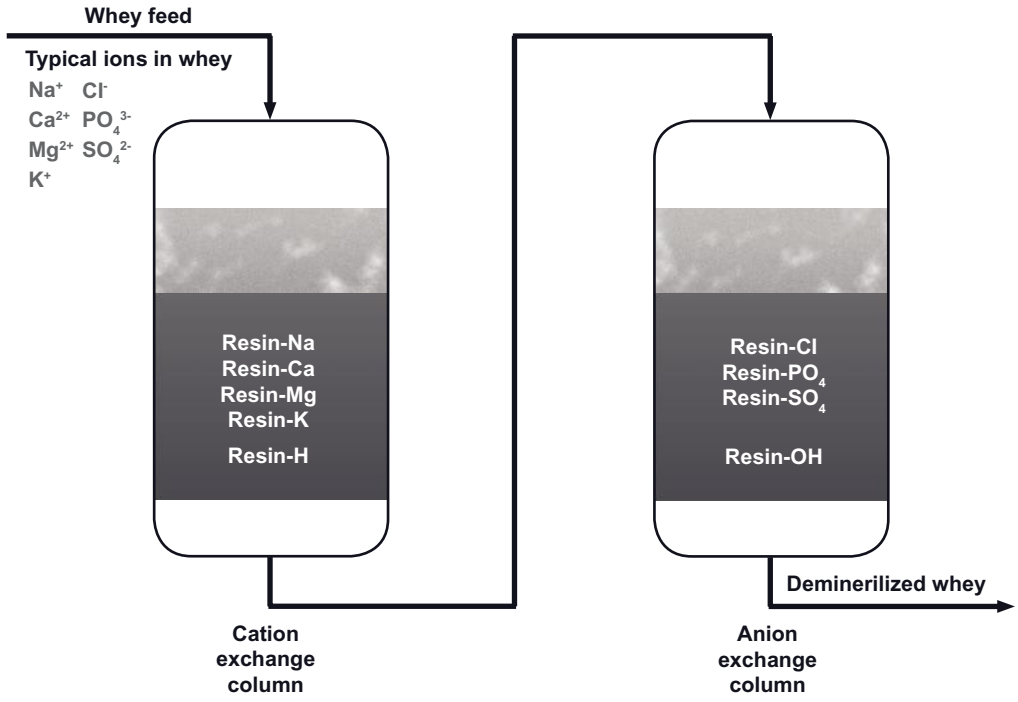

Figure 8.6. Ion exchange process.

using ion exchange chromatography processes, i.e., stirred tank processes (e.g., VISTEC and BiPRO processes), packed-column processes (e.g., Spherosil process), and continuous counter-current adsorption processes (e.g., ISEP contactor). Clogging and channeling in the adsorption stage are avoided in the stirred tank process. The disadvantage of the stirred tank process is that it cannot remove all the protein from whey and cannot equilibrate the bead with fresh whey. Both of these objectives can be achieved with packed-column processes. However, the counter-current adsorption processes are more efficient than either stirred tank or packed-column processes.

Ion Exchange (Demineralization)

In the ion exchange process of whey demineralization, resins of lower porosity are preferred because they have a higher selectivity toward smaller ions. According to Hoppe and Higgins (1992), whey demineralization is best carried out using strong acid cation resins and medium to weak base anion resins. Column systems are the most widely employed. Cation and anion exchangers are used either in mixed beds or in sequence. The stirred tank reactor leads to high resin attrition rates; most ion exchange resins are too fragile to maintain their physical integrity under these conditions.

As in the process described under ion exchange chromatography for protein isolation, the ion exchange process for demineralization is carried out in four stages: adsorption, washing, regeneration, and rinsing. If sulfuric acid is used as a regenerant, care must be taken to avoid fouling the system with calcium sulfate (Hoppe and Higgins 1992). Precipitation is controlled by using a progressive increase in acid strength during regeneration.

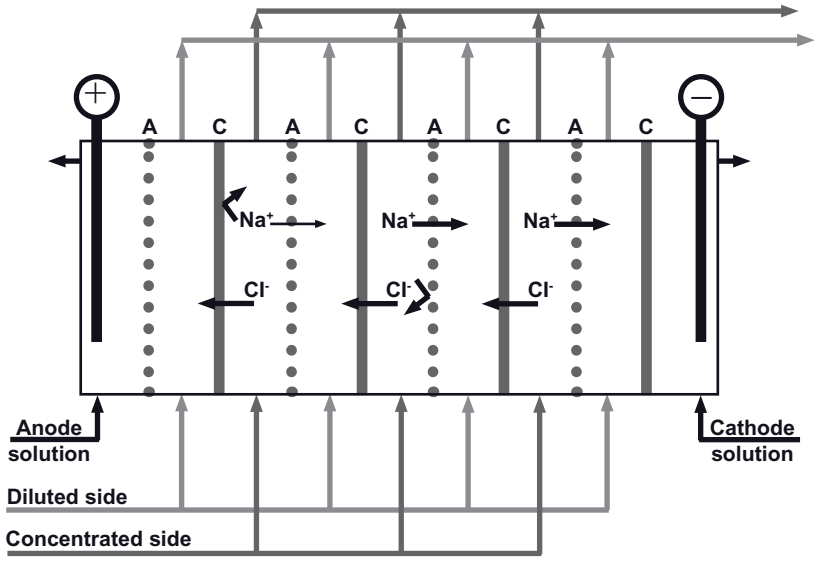

Figure 8.7. Electrodialysis process.

According to Hoppe and Higgins (1992), the following variables affect the process:

- The presence of agglomerates from cheese or casein manufacture may reduce the process efficiency.
- The feed concentration reduces the volume to be processed without reducing the demineralization load.
- Operating temperatures of 10°C (50°F) are often employed to reduce both the microbial growth in the system and the losses of protein and lactose by resin adsorption.

Electrodialysis

In electrodialysis the ionic components of a solution are transported through selective membrane barriers under the influence of an electrical potential. An electric field is formed between the anode (positively charged) and the cathode (negatively charged). Ion-selective membranes, made of similar material to ion exchange resins and with pore sizes of 10 to 20 Å, are stacked between the anode and the cathode, alternating cation and anion membranes to form a cell pair. Stacks can accommodate five to 500 cell pairs. As shown in Figure 8.7, whey is fed to alternate flow channels and the remaining channels, which are fed with brine, act as receivers for the ions being removed (Hoppe and Higgins 1992).

Whey should be concentrated to 20% to 30% solids prior to electrodialysis to make the process energy efficient (Kilara 2008). The system can be run in either batch mode or continuous mode depending on the degree of demineralization required. However, it is often accepted that 50% demineralization (which can be achieved by continuous processing) is commercially viable. At higher levels of demineralization, the high temperatures of the process (30°C to 40°C; 86°F to 104°F) and long times increase the chances of microbial contamination of the product.

Membrane fouling is a significant problem in electrodialysis. Both mineral fouling (calcium phosphate on the cation membrane) and protein fouling (anion membrane) can occur during processing. According to Kilara (2008), the replacement costs of membranes

Table 8.3. Reduction of minerals in whey, partially or fully demineralized by electrodialysis (ED), nanofiltration (NF), and ion exchange (IE).

Ions	60% ED reduction (%)	45% NF reduction (%)	40% ED reduction (%)	IE reduction (%)
K^+, Na^+	64	65	42	98
Ca^{2+}	35	6	24	92
Cl^-	89	54	71	95

Adapted from Hoppe and Higgins (1992), Bargeman et al. (2005)

are 30% to 40% of the total running costs of the process. Leakages at the gaskets between membranes may also cause significant losses of product. System improvements are continuously being developed to overcome these difficulties.

Table 8.3 highlights the differences between electrodialysis, nanofiltration, and ion exchange for the reduction of whey minerals.

Table 8.4. Typical composition of sweet whey powder and acid whey powder.

	Sweet whey powder	Acid whey powder
% Protein	12.8 ± 1.8	12.3 ± 1.3
% Lactose	69.0 ± 6.0	66.0 ± 4.0
% Fat	1.3 ± 0.3	1.0 ± 0.5
% Ash	8.5 ± 0.3	11.0 ± 1.3
% Moisture	4.3 ± 0.8	4.3 ± 0.8
% Calcium	0.7 ± 0.1	2.2 ± 0.2

Adapted from Renner (1992), Chandran (1997)

Whey Products and Ingredients

Drying Whey

Dairy products are dried primarily to extend storage stability, reduce transportation costs, and provide an ingredient that is easily incorporated in food formulations. Dehydration is an energy-intensive process. Water removal is generally more cost effective in an evaporator than in a spray dryer: a single-stage spray dryer requires six times the energy consumed by a conventional triple-effect evaporator (Goel et al. 1979).

Falling film evaporators are widely used for concentrating dairy products under vacuum. Whey ingredients may be concentrated to 30% to 60% solids with two or more stages. By adding more stages to the evaporator, the steam consumption/kilogram of water evaporated is reduced. In practice, the number of stages is determined economically by a balance of steam savings against capital outlay (Pearce 1992). Mechanical or thermal vapor compression may be introduced to further reduce evaporation costs.

Ingredients containing whey proteins range from whey powder with an average of 12.5% protein to whey protein isolates with a minimum of 90% protein.

Whey Powder

Whey powder is produced either by drying defatted fresh whey from rennet casein or cheese manufacture (dry sweet whey) or from cottage cheese, casein, or fresh cultured cheese types (dry acid whey). Sweet whey powder provides a mild and sweet flavor, whereas acid whey powder is a source of lactic acid flavor and dairy calcium. The increased content of calcium in acid whey is due to the colloidal calcium released from casein micelles during acidification. In general approximately 30% of the calcium in milk is in its free, ionic form. Table 8.4 gives the typical composition of sweet and acid whey powders.

The main steps in the processing of whey powder are as follows:

1. Cheese or casein fines recovery from whey. Cheese or casein fines impact negatively on fat separation and must be removed first (Bylund 1995). Centrifugal separators are commonly used.
2. Fat separation. Fat is recovered in centrifugal separators as whey cream containing 25% to 30% fat.
3. Concentration. Concentration is generally achieved by falling film evaporation under vacuum to a maximum of 65% solids. When energy costs are high, reverse osmosis is often considered as an option for the pre-concentration of whey (Storms et al. 1980). It will not fully eliminate the evaporator because it is limited to concentrations of less than 25% whey solids.
4. Crystallization. If whey concentrate is dried conventionally following evaporation, the final product will contain a large proportion of amorphous lactose. This leads to a very hygroscopic powder that may cake on storage. It is necessary to convert a high percentage of the lactose to its α-monohydrate crystalline form. Either lactose crystallization can be completed in the concentrate prior to drying or a two-stage dryer process that allows for crystallization prior to final drying may be used. When a crystallizer is used, the concentrate is cooled to 15°C to 20°C (59°F to 68°F) and kept under constant stirring for 6 to 8 hours to obtain the smallest possible crystals. Lactose crystallization is also promoted by rapidly cooling to less than 100°C (212°F) as the product is removed from the dryer (Morr 1992, Pearce 1992, Bylund 1995).
5. Drying. Whey is conventionally dried in a spray dryer. Care is taken with preheating conditions to ensure a good-quality product. According to Bylund (1995), acid whey from cottage cheese and casein is difficult to dry because it tends to form lumps. Neutralization, prior to drying, is therefore recommended.

Whey powders are used in bakery products, dry mixes, process cheese foods and spreads, frozen desserts, sauces, meat emulsions, confections, soups, gravies, snack foods, yogurts, and beverages (Chandan 1997).

Demineralized Whey Powder

Demineralization of whey began around 1985. Monovalent ions (Na^+, K^+, Cl^-) in whey powders result in negative sensory properties, whereas divalent ions (Ca^{2+}, Mg^{2+}) contribute to the health image of the product (Bargeman et al. 2005). The removal of minerals to various degrees is achieved with ion exchange, electrodialysis, and nanofiltraton. Typical demineralization levels are 50%, 70%, and 90%. With a demineralization level of 90% it is possible to produce a whey powder with a mineral content of less than 1%. Demineralized whey powders are ingredients in infant formula with a gross composition close to that of human milk.

Ion exchange is the most mature of the demineralization technologies. It has the ability to remove nearly all the minerals (both monovalent ions and divalent ions) present in whey and whey permeates (Hoppe and Higgins 1992). Electrodialysis is recommended for demineralization levels up to 50% to 60%. The process is ion-type selective, resulting in a higher loss of monovalent ions.

Nanofiltration is a relatively recent development. A crucial factor in the commercial viability of nanofiltration was the development of membranes with high lactose retention that allows effective salt removal. With nanofiltration the same level of demineralization as with electrodialysis is achieved, but at a lower rate of removal of divalent salts (Table 8.3) (Hoppe and Higgins 1992, Bargeman et al. 2005). An added advantage

of nanofiltration is the simultaneous removal of water, leading to a concentration of the product stream to 20% solids.

Whey Protein Concentrate

Whey protein concentrates (WPCs) are commonly produced by ultrafiltration. Lactose and minerals are removed while the proteins are concentrated. Different ratios of proteins : total solids can be obtained with the use of diafiltration (Figure 8.8).

WPC 35 is used as a partial or full replacement for skim milk powder and is also used in yogurt, bakery mixes, dietetic foods, and confections. WPC 50, WPC 65, and WPC 80 are used as protein supplements and are especially suited for use in nutritional drinks, sports and nutritional bars, soups, protein-fortified beverages, bakery products, and meat. WPC 80s also have good water-binding and thickening properties. Acid WPC 80s provide excellent gelling properties, and specialized WPCs are ingredients in infant formula. (Kilara 1994, 2008).

Whey Protein Isolate

Whey protein isolates (WPIs) have a minimum protein content of 90%. They are commonly produced either by defatting whey streams by microfiltration or by isolating native whey proteins by ion exchange chromatography followed by concentration by

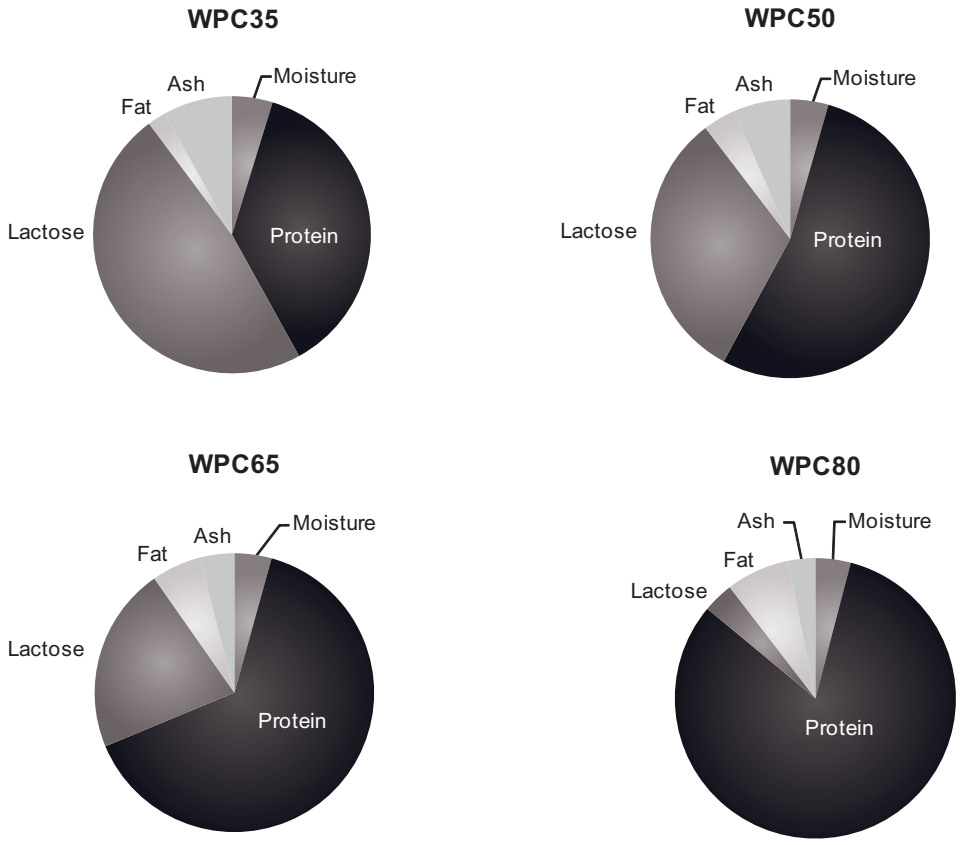

Figure 8.8. Typical composition of whey protein concentrates. Adapted from Mangino (1992), Huffman and Harper (1999), Kilara (2008), Abd El-Salam et al. (2009).

ultrafiltration, evaporation, and drying. The purification of whey proteins by microfiltration is based on the size difference between fat and protein. The isolation of whey proteins by ion exchange is based on the amphoteric nature of proteins; they can be adsorbed onto a porous, solid phase with a surface layer of charged groups, and then desorbed by an appropriate pH shift to lower the charge-charge attraction between the protein molecules and the ion exchanger (Ayers and Petersen 1985).

The difference between WPIs produced using microfiltration and those produced using ion exchange chromatography is given by their protein and mineral compositions. The main difference in protein composition relates to the absence of glycomacropeptides from WPI produced by chromatography (Table 8.5).

The isolation of whey protein by microfiltration directly from milk is recent, i.e., since 2000 (Marcelo and Rizvi 2008). This process produces a milk stream for whey-reduced cheese milk and cultured products and a WPI ingredient for beverages. Like the ion exchange WPI, this whey ingredient does not contain glycomacropeptides or peptide fragments derived from cheese or casein manufacture. However, the membranes allow for some casein transmission (Table 8.5).

Because of the relatively high costs of WPIs, they are used mainly in premium applications such as sports and nutritional supplements including beverages, sports gels, and nutrition bars.

Whey Fractions

Lactoferrin is a natural glycoprotein with iron-binding capacity. It is produced by means of ion exchange from either skim milk or whey. Because of its sensitive biological properties, it is normally freeze dried (Affertsholt and Nielsen 2007).

Lactoperoxidase is a natural preservative that has antimicrobial activity (Abd El-Salam et al. 2009). This fraction has yet to achieve the same commercial success as lactoferrin.

Immunoglobulins are transporters of antibodies against harmful microorganisms such as viruses and bacteria. The main source of commercial immunoglobulins is colostrum, a pre-milk fluid from the cow.

α-Lactalbumin is isolated from whey by chromatography. It is present in both human milk and bovine milk and as such is a beneficial ingredient in infant formula (Huffman and Harper 1999). It is a fully soluble protein and the main source of tryptophan, which is the precursor of neurotransmitters such as serotonin, norepinephrine, and dopamine. It is believed to help in regulating appetite, mood, and sleep (Affertsholt and Nielsen 2007).

Table 8.5. Typical protein and mineral composition of WPI.

Component	Microfiltration WPI	Ion exchange WPI
% α-Lactalbumin	15–22	14–26
% β-Lactoglobulin	56–60	66–75
% Bovine serum albumin	1–2	3–6
% Immunoglobulins	2–5	2–3
% Glycomacropeptides	20–26	Not detected
% Lactoferrin	0–0.1	Not detected
% Peptide fragments	3–5	Not detected
% Calcium	0.3–0.6	0.08–0.11
% Sodium	0.2–0.3	0–0.5

Adapted from Huffman and Harper (1999), Abd El-Salam et al. (2009)

Table 8.6. Definition of terms for protein functional properties related to food systems.

Property	Definition	Food System
Solubility	Ability to remain in solution over a broad pH range	Beverages, yogurt, salad dressings
Gelation	Ability to form a stable, cohesive gel when heated	Pumped meats, baked goods, cheese
Emulsification	Ability to keep two solutions that are not soluble in each other in a stable suspension	Infant formula, salad dressing, soup
Heat stability	Ability to remain in solution during heating	Infant formula, beverages
Whipping	Ability to form stable foams	Cakes, whipped desserts, ice cream
Water and fat binding	Ability to bind water, or entrap water, or bind free fat	Sausages, pumped meats
Nutrition	Protein amino acid profile and bioactivity	Infant formula, nutritional beverages, nutritional supplements

Adapted from Mangino (1992), Huffman (1996), de Wit (1998)

Functional Properties of Whey Protein Concentrates

According to Huffman (1996), whey protein products possess a number of functional and nutritional properties in varying degrees (Table 8.6). However, no single whey protein can be used in a wide spectrum of food products because very few proteins exhibit the needed multiple functionalities (Abd El-Salam et al. 2009). Mangino (1992), Kilara (2008), and Abd El-Salam (2009) have reviewed and assessed the functional properties of a variety of whey protein ingredients and the processes to manufacture those ingredients.

Solubility

Solubility is probably the key property of most whey ingredients. If the product is not soluble, the other properties are usually also poor.

Whey proteins demonstrate excellent solubility over a wide pH range. Because most food systems have pH values in the range of 3 to 7, it follows that whey protein ingredients will remain soluble in practically all liquids and most foods unless heated. The advantage of whey proteins over other protein systems, e.g., caseinates and soy protein isolates, is that caseinates and soy protein isolates have poor solubility below pH 4.5 and so are less suitable for low-pH foods if solubility is a requisite.

The solubility of proteins is lowest at their isoelectric pH (when the protein has a net charge of zero). Because the overall isoelectric pH of the whey proteins is in the region of pH 5, there is a slight dip in the solubility of whey proteins at pH 5 (Kilara, 2008).

One particularly important factor affecting the solubility of protein products is the level of whey protein denaturation. The denaturation of whey proteins is generally a function of the amount of heat treatment that the protein has received, although other factors such as shear and foaming are also relevant (Mangino et al. 1988).

Heat treatments have an additive effect on whey protein denaturation, with temperatures as low as 55°C (131°F) leading to denaturation over time. Therefore, good control of upstream processing is very important (Patel et al. 1990). Very small amounts of whey protein denaturation lead to enhanced water and fat binding by WPCs. Such products may present enhanced viscosity, lower gelation temperatures, enhanced emulsification, and cold setting (Schmidt et al. 1984, Abd El-Salam et al. 2009).

Factors affecting whey protein denaturation include:

- Temperature-time profile during processing
- pH
- Total solids
- Ionic strength and composition

Gelation

Under appropriate heating conditions, whey proteins form irreversible gels by restructuring into extended three- dimensional networks.

Heat gelation of whey proteins is generally recognized as a two- or three-stage process: denaturation of the native globular structure into a partially unfolded state, followed by aggregation of the denatured protein into a gel matrix, which can bind large amounts of water (De la Fuente et al. 2002b, de Wit 2009). However, a delicate balance between attractive and repulsive forces is required to give the desired gel.

Whey proteins start to gel when heated to around 65°C (149°F). A strong gel network helps to hold the water and prevent moisture loss (syneresis). This property improves the yield value of various food products (e.g., ham) and can also improve the appearance by preventing surface moisture (e.g., yogurt).

Emulsification

The formation of emulsions is important in a number of applications in which fat or oil is present (e.g., salad dressings). Whey proteins are candidates for emulsification because the proteins have both hydrophobic regions and hydrophilic regions. Whey proteins are thought to form interfacial membranes around oil or water globules, preventing creaming, coalescence, and oiling off. After adsorption at the fat or water interface, the protein partially unfolds to stabilize the globules. Whey proteins maintain their solubility under acidic conditions; therefore, they can perform well where many other proteins cannot (Fachlin and Viotto 2005). Whey protein ingredients also are useful in low-fat foods to provide the body and mouth feel of fat without the addition of fat.

Heat Stability

Many whey protein ingredients are used in retorted nutritional products (infant or enteral formulas). These products are usually given heat treatments of about 120°C (248°F) for 15 minutes to sterilize them, allowing a shelf life of around two years for some products. The ability of the proteins to stay in solution during the heating step is crucial, requiring specific treatments to the complex formulations.

Foaming and Whipping

Foaming of protein solutions can be desirable in some applications (e.g., aerated products such as desserts and meringues) but is undesirable in other applications (such as fruit juice fortification or in the brine pumping of hams).

The formation of foam is a multi-stage process involving denaturation of protein, adsorption to the air/water interface, entrapment of air, repair, contact-stabilization, and coagulation-destabilization of the foam.

The speed of air incorporation and the stability of the foam depend on parameters such as whey protein type, protein denaturation level, fat content, protein and carbohydrate concentrations, concentrations of calcium and other ions, pH, and equipment (Morr and Foegeding 1990, Farrag 2008). The presence of fat is particularly detrimental to the formation of foams due to the hydrophobic disruption of the surface tension of the film, and whey protein ingredients with reduced fat content such as WPIs show the best performance where foam formation is required.

Viscosity and Water Binding

The viscosity of whey protein ingredients in water is low, which allows the use of

high-protein concentrations, thus reducing the need to consume large volumes of liquid to get the required protein intake. Heating whey proteins causes an increase in viscosity and an increase in water-binding capacity, but a potential decrease in solubility (de Wit 1989). In addition, the formation of protein aggregates increases the volume occupied by the protein, which is a key contributor to the increased viscosity.

Nutrition

Milk proteins provide an excellent source of high-quality protein (Ha and Zamel 2003). There are many different means of assessing protein quality. World health authorities (Food and Agriculture Organization/World Health Organization; FAO/WHO) have established an amino acid profile that reflects the daily requirements of essential amino acids to ensure maintenance of good health. By comparing the amino acid content of a protein with this ideal composition, it is possible to estimate the extent to which the product fulfills the body's requirements for essential amino acids (Table 8.7).

The amino acid score based on the FAO/WHO requirements is combined with the protein digestibility measurement to give the PDCAAS (protein digestibility corrected amino acid score). Protein digestibility is now the most commonly used biological method for measuring protein quality, replacing the PER (protein efficiency ratio). The whey proteins meet the minimum requirements for essential amino acids.

Whey proteins contain high levels of the branched chain amino acids leucine, isoleucine, and valine. These amino acids can be metabolized as an energy source and thus are considered to be useful in sports drinks, where they assist both as an energy source and as a protein source, and for muscle building or sparing (Ha and Zamel 2003).

It is also relevant to consider the lactose content of ingredients, especially where there is a risk of a final product being fed to lactose-intolerant people. Recent developments in whey processing, with the use of ion exchange and microfiltration for whey protein isolation, have led to the generation of ingredients with less than 1% to 2% lactose that are suitable for reduced-lactose and lactose-free end products, depending on their formulation.

Biological Properties

There is mounting evidence relating to the potential health benefits of whey and its components. At present most of the data come from *in vitro* and *in vivo* animal studies, with limited human clinical trials. Table 8.8 summarizes the current trends (Harper 2000, 2001). However, to date there has been minimal commercial exploitation of this knowledge.

Table 8.7. Preschool age amino acid requirements set by FAO/WHO (1989).

Essential amino acid	Recommended requirement (mg/g protein)	WPC 80 (mg/g protein)
Isoleucine	28	54
Leucine	66	119
Lysine	58	94
Methionine + Cysteine	25	52
Phenylalanine + Tyrosine	63	68
Threonine	34	66
Tryptophan	11	20
Valine	35	51
Histidine	19	21

Table 8.8. Potential health benefits of whey products and whey components.

Health benefit	Component
Immunomodulation	Lactoferrin Lactoperoxidase κ-Casein glycomacropeptide
Protection from some cancers	WPC Lactoferrin α-Lactalbumin Peptides Sphingolipids
Protection from hypertension	Peptides
Anti-inflammatory	Peptides Colostrum
Anti-thrombotic activity	Lactoferrin Peptides
Reduction in cholesterol level	WPC
Opioid-like activity	Peptides
Prebiotics	Casein glycomacropeptide Oligosaccharides
Anti-oxidative effects	WPC/WPI Lactoferrin Bovine serum albumin

Adapted from Harper (2001)

Lactose Processing, Products, and Derivatives

Lactose is crystallized from whey or milk permeates obtained by ultrafiltration processing. Lactose processing is similar to whey powder processing in that it involves the following:

1. Reverse osmosis of the whey permeate to 20% to 25%. Often this process is carried out under slightly acidic conditions to reduce the fouling caused by calcium phosphate salts.
2. Evaporation to 50% to 60% solids. Generally, falling film evaporators are used in this step of the process. The concentration that can be achieved depends on controlling scale formation caused by calcium salts (Harper 1992).
3. Crystallization followed by centrifugation to separate the crystals. Impurities are washed from the lactose during separation. The residue is called mother liquor and is used as feedstock. The degree of crystallization is determined by the quantity of β-lactose that is converted to the desired α-lactose form. Crystalline α-lactose hydrate is hard and not very soluble; β-lactose crystals are sweeter and more soluble (Chandan 1997). According to Harper (1992), the following factors are relevant in crystallization:
   a. Original concentration of the lactose in the solution
   b. Concentration of the lactose in the solution after crystallization has taken place
   c. Time and temperature of cooling
   d. Level of impurities in the concentrate
   e. Viscosity of the concentrate
   f. Seeding material

   The following factors are relevant to separation (Harper 1992):
   a. Homogeneity of the crystals
   b. Viscosity

   c. Amount of washing required (proportional to the level of impurities)
   d. Centrifuge design; the decanting centrifuge is commonly used today
4. Drying of the crystals to a powder; the temperature during drying should not exceed 93°C (199°F) because β-lactose is formed at higher temperatures (Bylund 1995). Drying times are also important to avoid the formation of amorphous lactose on the α-hydrate crystal.
5. The refining of lactose for higher grade applications such as pharmaceuticals. This involves rehydration in hot water to 50% solids and filtration in the presence of active carbon, phosphate, and a filtration agent; followed by crystallization, separation, and drying. An alternative procedure is to use ion exchange to remove mineral impurities from the crude lactose (Harper 1992).
6. Grinding. The size of the lactose crystal is relevant to the application.

Lactose is available in various grades: crude lactose (purity between 95% and 98%); edible and refined edible grade (99% to 99.5% purity); pharmaceutical grade (99.5% to 99.9% purity).

Lactose is used in a variety of foods: baked goods, dry soups and sauces, infant formulas, meat products, confectionery, and drinks. It is used for reduction in sweetness, fortification of aroma, improved color of baked products (because lactose is not fermented by Baker's yeast, its functional properties are not lost during the fermentation stages of the process), and improved shelf life. The delayed crystallization of concentrated sucrose solutions when lactose is added is useful in the coating of candies (Harper 1992).

In the pharmaceutical industry, lactose is used as a carrier for tablet making (where flow and compressibility are the key properties), as a carrier in dry powder inhalers, and as a filling agent in capsules.

In the pursuit of increased value from lactose, the generation of derivatives from lactose is of interest to the dairy industry. The most relevant derivatives are enzymatic hydrolysis products of lactose, lactulose, tagatose, lactitol, lactobionic acid, lactic acid, and galactooligosaccharides. Only a few of these products have found commercial relevance in the dairy industry. The enzymatic conversion of lactose into glucose and galactose in dairy products generates products that are suitable for people suffering from lactose intolerance. Lactulose is manufactured in large scale from lactose by alkaline isomerization; its main application is in medicine as a constipation regulator, a growth promoter of bifidobacteria, a treatment of hepatic encephalopathy, and a treatment of salmonellosis (Affertsholt and Nielsen 2007). Galactooligosaccharides are carbohydrates comprising 2 to 10 monosaccharide units; they can be produced by the same enzyme that hydrolyzes lactose. Tagatose is a naturally occurring ketohexose that has been suggested as a low calorie sweetener. Despite large investments in research and development and a commercial plant, production by Arla was halted in 2006 (Affertsholt and Nielsen 2007).

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Chapter 9

Butter and Butter Products

Anna M. Fearon

Introduction

Milk fat is used in a variety of forms in the food processing industry. It may be included as cream, butter (salted, unsalted, or cultured), in a concentrated form such as anhydrous milk fat, as milk fat fractions, or as a component of a blend with other fats. The functional characteristics of milk fat in its different forms are important in the wide variety of applications for which it may be used. These characteristics include milk fat's unique and desirable flavor, its plastic fat properties wherein milk fat (or butter) displays solid behavior over a range of temperatures, its relatively high oxidative stability in comparison with vegetable oils, and its ability to melt completely in the mouth. In recent years manufacturers of milk fat products have been able to tailor their composition and properties to more closely meet the performance customers require within product applications.

Butter Manufacture and Properties

Council Regulation (EC) 2991/94 and Council Regulation (EC) 1898/87 (protection of dairy designations) describe how the term butter may be used for labeling purposes. "Butter" is reserved for a product with a milk fat content of not less than 80% but less than 90%, a maximum water content of 16%, and a maximum dry nonfat material content of 2%. The dairy designation regulations allow only milk fat as the fat source within such a product. Exceptions regarding use of the term are permitted where traditional usage of the term "butter" has applied to a characteristic of the product, for example, "peanut butter" and "cocoa butter" (Council Regulation (EC) 1898/87). "Butter" may also be applied to composite products in which the end product contains at least 75% milk fat and an essential part of the end product, in terms of quantity or characterization, is butter. Regulations defining butter composition and labelling in other major butter-producing countries such as New Zealand and the United States are similar to those described in the Council Regulations (EC) 1898/87 and 2991/94 (Table 9.1).

Principles of Butter Making

The manufacture of creamery butter is the basis for production of most of the concentrated milk fat products; therefore, it is useful to consider the science and technology underpinning the process. The main steps in the production of creamery butter are common, whether carried out in a batch or continuous butter maker, and are summarized below:

- Preparation of cream by centrifugal separation of liquid milk to a fat content typically about 40%

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Table 9.1. EC Sales descriptions of "spreadable fats."

Fat content ranges	Sales descriptions		
	Milk fat products	Vegetable/animal fat products	Mixed milk fat and vegetable/animal fat
Equal or more than 80% but less than 90%	Butter	Margarine	Blend
More than 62% but less than 80%	Dairy spread X%	Fat spread X%	Blended spread X%
Equal to or more than 60% but less than or equal to 62%	Three-quarter fat butter Reduced fat butter	Three-quarter fat margarine Reduced fat margarine	Three-quarter fat blend Reduced fat blend
More than 41% but less than 60%	Dairy spread X% Reduced fat dairy spread X%	Fat spread X% Reduced fat spread X%	Blended spread X% Reduced fat blended spread X%
More than or equal to 39% but less than or equal to 41%	Half-fat butter Low-fat butter Light butter	Half-fat margarine Minerine Halverine Low-fat margarine Light margarine	Half-fat blend Low-fat blend Light blend
Less than 39%	Dairy spread X% Low-fat dairy spread X% Light dairy spread X%	Fat spread X% Low-fat spread X% Light-fat spread X%	Blended spread X% Low-fat blend X% Light blend X%

Adapted from Annex of EC Council Regulation 2991/94. Source: Fearon and Golding (2008)

- Cream aging to promote crystallization of milk fat using selected temperature regime(s)
- Emulsion destabilization and phase inversion from an oil/water cream emulsion to water/oil butter emulsion achieved by physical agitation (churning)
- Physical working of butter grains to form larger granules, expel buttermilk, distribute moisture, and create a homogeneous butter mass

Butter Making Technology

In recent years much of the development in butter making technology has been in improving the efficiency of the equipment to reduce butterfat losses, increase buttermilk drainage, increase butter yield, reduce power consumption, and ultimately improve profitability. Butter technology and manufacture have been described by Fearon and Golding (2008).

Cream

Cream of the required fat content is prepared by separating liquid whole milk into skim milk (0.05% fat) and cream (e.g., 38% to 42% for most continuous butter makers) in a centrifugal separator. Centrifugal separators use the density differences between the milk fat globules and the aqueous serum phase. Plants may operate a cold separation procedure (below 10°C; 50°F) to reduce free fat loss into the skim milk, although a higher separation efficiency is achieved as temperatures, and density differences, increase. Cold separated cream contains higher quantities of phospholipids, which improves whipping properties.

The cream is then pasteurized in a continuous high-temperature short-time (HTST) plate heat exchanger, normally to a higher temperature than milk pasteurization, before cooling to aging temperature. The minimum pasteurization temperature/time combination recommended is 72°C to 77°C (161.6°F to

170.6°F) for 15 seconds for most countries, but higher temperatures up to 95°C (203°F) are frequently used. Flavor taints from animal feed can be removed at the cream pasteurization stage by carrying out the heat treatment under vacuum (vacreation) before cooling the cream. Vacreation, however, can increase fat loss into the buttermilk.

Cooling and aging of cream are important because this part of the process affects the number and size of fat crystals formed and ultimately affects the spreading characteristics of the butter. Cream for traditional sweet cream butter is cooled after pasteurization to 5°C to 10°C (41°F to 50°F) to encourage nucleation of the fat crystals and then aged at a similar temperature for 7 to 12 hours before churning, usually around 10°C to 13°C (50°F to 55.4°F). The appropriate cream tempering regimes to modify fat crystal size and number, and hence spreading properties, can be selected based on melting and solidification curves for milk fat (Precht et al. 1981) or by identifying hard and soft milk fats from iodine values.

The cold-warm-cold cream tempering procedure was developed to improve the spreadability of hard winter butter (Figure 9.1). An initial low temperature, for example, 5°C to 8°C (41°F to 46.4°F), causes the rapid formation of many small milk fat crystals, leading to a high solid fat content with less liquid oil available due to its entrapment within the crystal network and adsorption at the crystal surfaces. When the cream is gently heated in the warm phase (14°C to 21°C; 57.2°F to 69.8°F), partial melting of these crystals takes place, followed by a period of slow reformation of high-melting-point fat into larger crystals. Reducing the temperature again (second cold phase) causes some additional crystallization of lower melting point fractions, resulting in the growth of larger crystals, but with minimal entrapment of liquid oil within the fat crystal structure. By maximizing the relative amount of liquid oil within the emulsion, a softer butter can be produced.

More recently, fractal studies (studies of crystal microstructure geometry and dimensions) have been employed to determine quantitative parameters that characterize the milk fat crystal microstructure, which changes in response to processing variables. These parameters can then be used to predict the texture of butter and may be applied by butter manufacturers to control processing conditions such as cooling rate to derive the

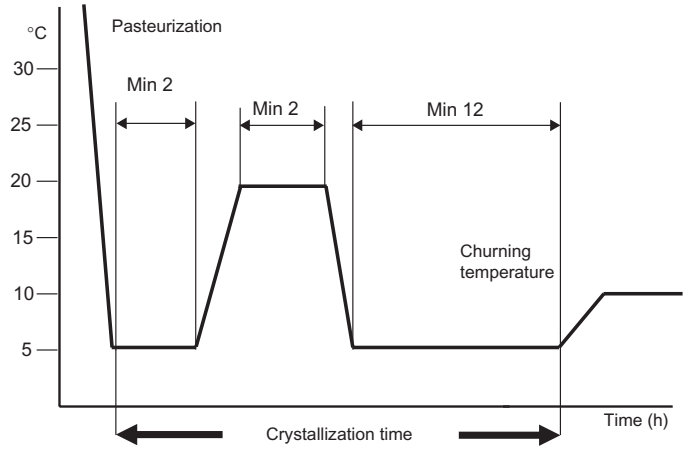

Figure 9.1. Temperature-time curve for cold-warm-cold treatment of cream. Adapted from GEA Westfalia Separator, Germany.

Table 9.2. Principal fatty acids in milk fat (g fatty acid/100 g total fatty acids).

Fatty acid (Carbon number)	Winter butter*	Summer butter*
C4:0	2.9	2.2
C6:0	1.7	1.5
C8:0	1.3	9.6
C10:0	2.9	2.0
C12:0	3.3	2.4
C14:0	11.5	9.7
C14:1*c*	1.4	1.2
C16:0	33.0	26.6
C16:1*c*	1.9	1.7
C18:0	11.0	12.6
C18:1*c*9	22.5	28.7
C18:1*t*	2.2	3.6
C18:2*c*9,12	1.3	0.86
C18:2 conjugated**	0.5	1.6
C18:3*c*9,12,15	0.8	1.4

*Winter diets of concentrates and silage; summer diets of mainly fresh grass
**Total conjugated linoleic acid, principally C18:2*c*9,*t*11 isomer

desirable product properties (Wright et al. 2001).

The proportion of solid fat in butter is highly correlated with product firmness and strongly influenced by the cows' diet and stage of lactation. The fatty acids in milk fat (Table 9.2) originate from two main sources: those acids synthesised *de novo* in the mammary gland, C4-C14 and a proportion of C16 acids, and those arising directly from the diet and taken up by the mammary gland from the circulating blood, i.e., the remainder of C16 and the longer-chain C18 acids. When the dairy cows graze fresh pasture, this leads to a softer, more unsaturated milk fat with a reduction in content of saturated fatty acids, especially C16:0, and an increase in monounsaturated fatty acids, mainly C18:1. The reverse occurs during winter feeding of concentrates and silage.

The cooled cream may be held in cream aging tanks or, for larger operations, in silos. Slow-moving agitators or intermittent mixing are necessary to prevent separation, but care should be taken to avoid aeration or damage to the fat globules.

Ripened cream may be produced at this stage for cultured butter. Usually such cream is pasteurized at a higher temperature than cream for sweet cream butter, cooled to ripening temperature (20°C to 27°C; 68°F to 80.6°F), and inoculated with starter culture (1% to 2%). Normally a mixed culture of lactic microorganisms, for example, *Lactococcus lactis* subsp. cremoris, *Lactococcus lactis* subsp. lactis, and *Lactococcus lactis* biovar. diacetylactis, is added to the cream to ensure acid (pH 5.3 to 4.7) and flavor (especially diacetyl) development. The primary aroma producers are *Lactococcus lactis biovar*. diacetylactis and *Leuconostoc mesenteroides* subsp. cremoris. Cooling the cream controls the extent of the fermentation process and strength of flavor development, and allows crystallization of the fat in the cream to be completed before churning.

An alternative process developed in the mid 1970s by the Netherlands Dairy Research Institute is the NIZO method, which involves adding a mixture of cultured whey concentrate and bacterial culture to sweet cream butter during working. The concentrates, which can vary in composition, add lactic acid, aroma, and flavor compounds to butter, thus avoiding the production of lactic buttermilk. The advantages of using concentrates to produce ripened or cultured butter are that starter cultures need not be stored or prepared in a factory laboratory, and disposal of sweet cream buttermilk is both easier and cheaper than disposal of lactic buttermilk.

Butter Formation

Butter is now commonly manufactured using continuous butter making machines. These machines have the advantage over the older batch churns (Figure 9.2) in terms of consistent production of a butter of uniform quality with low air content (better texture and less oxidation), improved moisture distribution, and smaller water droplet size (improved

shelf life and bacteriological quality). The impact of butter making technology on the microstructure of butter has been described in detail by Fearon and Golding (2008).

A continuous butter making machine from APV Unit Systems, Denmark, is shown in Figure 9.3; the capacity of such equipment ranges from 500 kg/hour to 12,000 kg/hour, and their flexibility enables sweet, cultured, or whey creams with a range of fat contents to be churned, as well as dairy blends of cream mixed with vegetable oil. Greater operating detail may be seen in the schematic outline of the machine in Figure 9.4. The machine is divided into three sections: churning, separating, and working.

Figure 9.2. Batch butter churn (APV Unit Systems, Denmark). Fearon and Golding (2008).

Churning Section

The churning section consists of a horizontal cylinder and a multi-bladed beater that sits only a few millimeters from the cylinder wall. Aged cream at the desired churn temperature is pumped into the rear end of the churning cylinder, where the beater, operating at speeds of approximately 1,000 to 1,500 rpm, introduces air into the cream, damages the fat globule membrane, and causes the globules to agglomerate. Selection of beater speed and speed constancy is important to achieve lowest fat loss into the buttermilk and highest moisture content of the buttermilk. The size and moisture content of the butter grains as well as the fat content of the buttermilk are largely determined by the speed of the beater. Butter grains must be of a size to allow good drainage of the

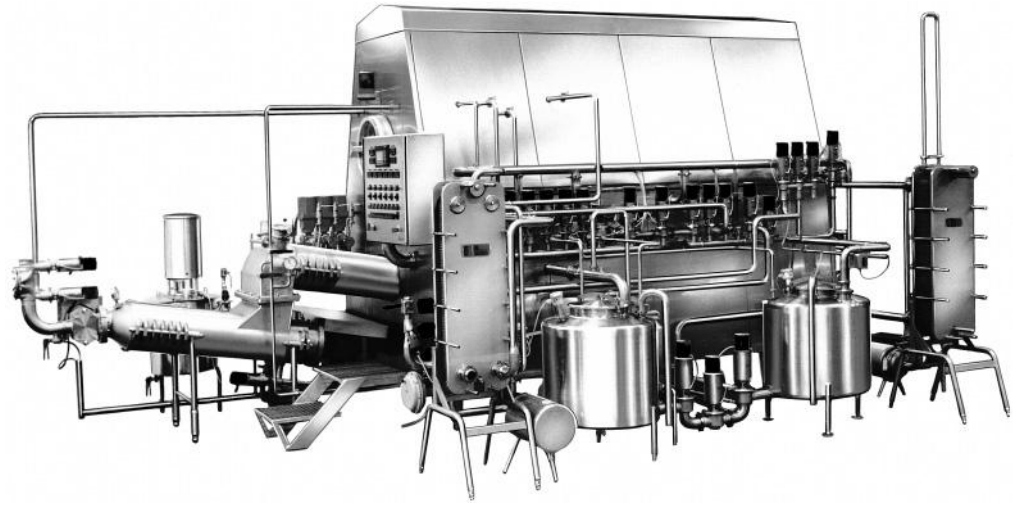

Figure 9.3. Continuous butter making machine (APV Unit Systems, Denmark). Fearon and Golding (2008).

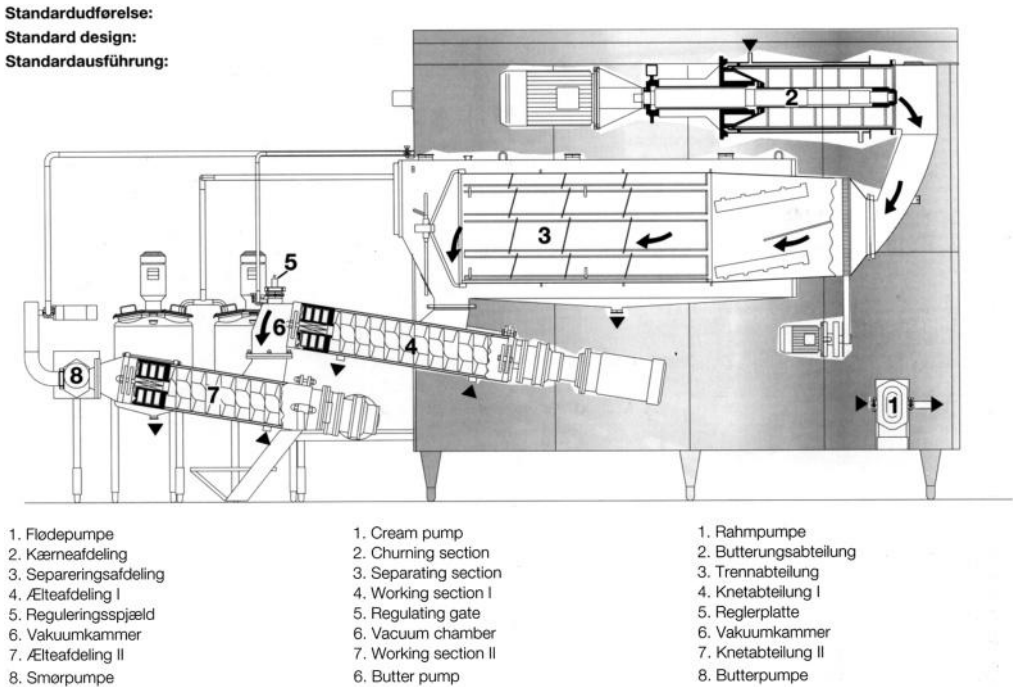

Figure 9.4. Schematic outline of a continuous butter making machine (APV Unit Systems, Denmark). Fearon and Golding (2008).

buttermilk; excessively large or small grains retain too much moisture. The churning process only takes a few seconds and a mixture of butter grains and buttermilk then flows into the separating chamber.

Separating Section

The separating section consists of a horizontal rotating cylinder in which two operations are performed. The first part of this section contains beaters to continue churning the mixture of buttermilk and butter grains, causing larger clumps to form and more buttermilk to be expelled. A perforated filter, or separation drum, then separates the buttermilk from the butter, while the rotation of the drum encourages further clumping of the grains. Operating parameters of the churning and separating sections such as temperature and churning/rotating speed are controlled to meet cream and product requirements.

Working Section

Typically there are two working sections linked by a vacuum chamber in butter making machines. Working section 1 comprises augers for transportation of the butter and also working elements, i.e., working vanes and perforated plates. The butter mass is kneaded in this section, expelling buttermilk before addition of water and/or salt slurry. If the augers operate at too low a speed they will not squeeze sufficient buttermilk out of the butter.

Butter is forced through a regulating gate between working sections 1 and 2. By adjusting the apertures of the gate, the counter pressure on the butter can be adjusted and hence

the amount of buttermilk expelled can be regulated. Passing through the apertures of the regulating gate greatly increases the surface area of the butter; however, by applying a vacuum in the chamber connecting the two working sections it is possible to reduce the air content in the butter from 5% to 6% to less than 0.5%. Deaeration improves shelf life and appearance of the butter, resulting in a more closely textured product than is found in traditionally worked butter.

Working section 2, like the first working section, consists of augers and working elements; however, the auger speed is usually two or three times higher than in working section 1. The function of the second working section is to carry out the final working of the butter and ensure that water and salt are evenly distributed throughout the butter, with water droplet size as small as possible, approximately 5 microns, to prevent undesirable microbial growth during storage. Overworking, however, produces a sticky butter that is difficult to pack. Salt is introduced into butter in modern equipment as a slurry via a computer-controlled pump. The slurry is a mixture of ultra fine salt grains (less than 20 microns) and water in a 50:50 ratio. It is important that all salt particles are dissolved in the moisture in the butter by the end of working, otherwise color and flavor defects will occur in the final product.

Packing

Butter may be packed in bulk in 25-kg polythene-lined cardboard cartons for chill or frozen (-18°C or -25°C; -0.4°F or -13°F) storage, printed as 250-g or 500-g retail blocks, and wrapped in parchment or lined foil, or extruded into attractive plastic tubs similar to those used for margarines and other spreadable fats. Re-packing bulk butter for retail purposes usually requires reworking the butter first. The temperature of the bulk butter is raised to 5°C to 8°C (41°F to 46.4°F) either by holding it in a store at this temperature or by using microwave tunnel heaters. It is then worked to blend and standardize the salt and moisture content of the final product. An alternative process involves chopping the frozen butter into thin strips while maintaining the butter temperature at 0°C to 2°C (32°F to 35.6°F). The butter is then worked in the blender section several times and deaerated before being packaged in retail units. It is important to note that butter is quite soft when it first emerges from the churn, but it will set or harden gradually over a period of about four weeks. Freezing interrupts the setting process, but setting resumes when the butter is removed from frozen storage. Re-working butter such as takes place with bulk stored butter before retail packaging also softens butter by disrupting the three-dimensional structure. Recovery of hardness, however, occurs during subsequent storage at refrigerator temperatures.

Low-fat Butter

The traditional process for producing low-fat or half-fat butter (40% fat) is based on using butter oil into which an aqueous phase such as milk or buttermilk and stabilizers, emulsifiers, colorings, flavors, and antioxidants are blended. This mixture is then chilled and crystallized in a scraped surface heat exchanger (see the section on spreads). An alternative process (APV patented method) may be employed that uses standard butter directly (Figure 9.5). In this process the butter is worked and deaerated in a vacuum working section, where it is also heated gently before being pumped into a butter homogenizer. The softened butter is first dosed with a pasteurized solution of sodium caseinate before the mixture is homogenized to ensure that a homogeneous blend of the butter and protein solution is achieved, with small (approximately 5 microns) well dispersed water droplets. The degree of homogenization depends upon the milk fat composition of the original

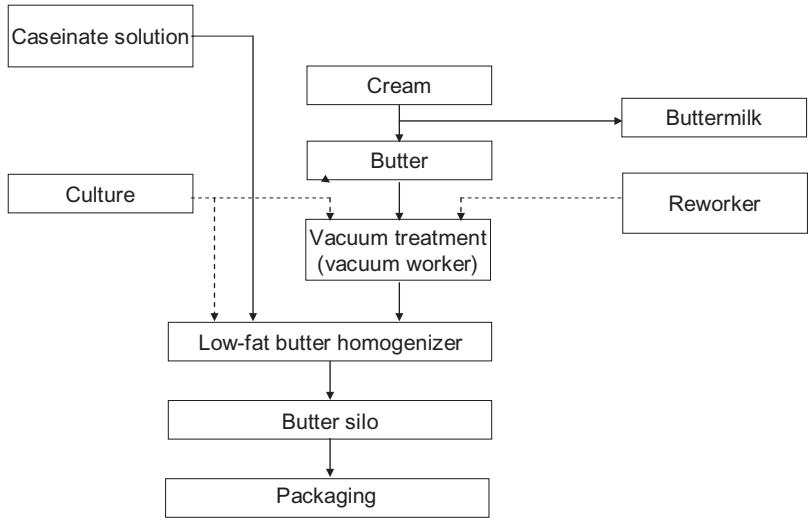

Figure 9.5. Low-fat butter production (APV Unit Systems, Denmark). Fearon and Golding (2008).

butter, homogenization temperature, and the required fat content of the finished product. Starter culture and salt can also be added at this stage. Following homogenization, the blend is pumped into a silo and from there to a scraped surface heat exchanger for cooling before packing.

Spreadable Butter

Butter is recognized as a high-quality natural product with a unique flavor; unfortunately, it is also well known that butter is hard and virtually unspreadable at refrigerator temperature, and this property makes it compare unfavorably with dairy spreads and table (tub) margarines. The primary reason for the firmness of butter is the high content of saturated fatty acids in milk fat that are solid at low temperatures (Table 9.2). Legislation prohibits any fat other than milk fat to be present in butter, which limits options available to butter manufacturers to improve butter spreadability. As mentioned earlier, physical working to disrupt the three-dimensional butter structure or application of a cream-tempering regime to alter fat crystal number and size can achieve moderate success. However, greater success has been achieved in recent years by modifying the fatty acid composition of the milk fat through changes to the dairy cow's diet.

Many reports in the literature concern modification of the cow's diet to increase the content of unsaturated fatty acids in milk fat, thus reducing solid fat content. These have been reviewed by Ashes et al. (1997) and more recently by Murphy (2000). Because the long-chain fatty acids in milk fat, i.e., all of the C18 acids and approximately 50% of C16 acids, originate from the cow's diet, they have been the focus for dietary manipulation of milk fat. In a cow's digestive system (rumen), microorganisms normally hydrolyze and hydrogenate dietary lipid, mainly resulting in saturated and partially saturated fatty acids entering the blood stream to be transported to the mammary gland, where milk is synthesized. In the mammary gland, the delta-9 stearoyl desaturase enzyme then converts a significant proportion of the saturated C18:0 fatty acid, stearic acid, into the monounsaturated C18:1 fatty acid, oleic acid.

"Pure" naturally spreadable creamery butter was first launched under the Dromona label by Dale Farm Limited in Northern Ireland in 1999 (launched under the "Pure" brand name in 2003), and is one of the few dietary modified spreadable butters that has made it onto the commercial market. Dairy cows are offered a concentrate containing whole rapeseed during the summer months, when the animals graze fresh grass. The combination of partially protected rapeseed and fresh grass results in milk fat with a high content of unsaturated fatty acids, in particular C18 : 1 oleic acid, and an iodine value of more than 45 g iodine/g fat (Fearon et al. 2004). The sweet creamery butter has markedly improved spreadability at low temperatures while maintaining product body at room temperature.

Previous spreadable butters have been produced by feeding large amounts of rumen-protected polyunsaturated oils to dairy cows, which substantially increased the content of polyunsaturated fatty acids in the milk by bypassing rumen hydrogenation. However, rapid oxidation of the raw milk and butter was a major problem (Banks and Christie 1990). The main unsaturated fatty acid in Pure butter is a monounsaturated acid (oleic acid), hence oxidation is not a problem (Fearon et al. 2004). The product commands a premium price, while milk producers also receive an increased payment to compensate for higher feed costs and reward them as members of the scheme.

An alternative approach to produce spreadable butter within butter labeling regulations has been to incorporate a lower-melting fraction of milk fat, or a blend of milk fat fractions, into butter or cream. Solid fat content in milk fat from pasture-fed dairy cows may be as high as 50% at refrigerator temperatures, while butter produced under winter feeding conditions (conserved forage and concentrates) will be higher still (Figure 9.6). In order to achieve optimum spreadability in a spreadable fat product at 5°C (41°F), it would be necessary to reduce the solid fat content to 30% to 40%, but this must be balanced by a solid fat content of 10% to 20% at room temperature (20°C; 68°F) to support product body and prevent oiling off. Blends of milk fat fractions with different melting points seem to be preferred over

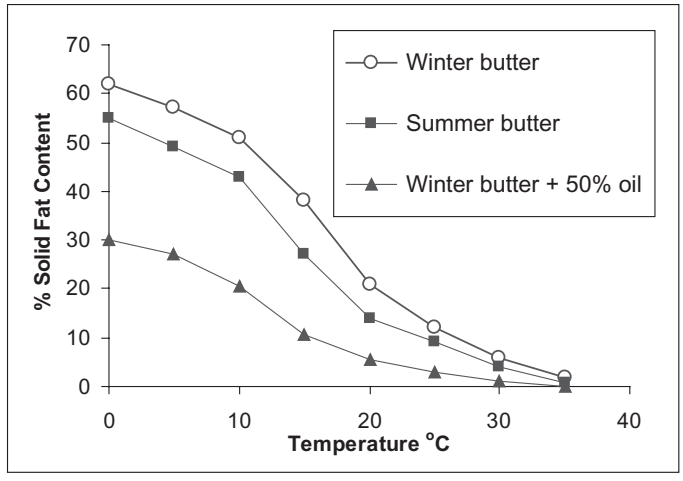

Figure 9.6. Solid fat content vs. temperature curves for winter butter, summer butter, and a blend of winter butter and 50% vegetable oil (measured by nuclear magnetic resonance). Adapted from Fearon 1986. Fearon and Golding (2008).

addition of a single low-melting fraction to achieve these properties in the final butter product (Kaylegian and Lindsay 1992).

Dairy Spreads

It is apparent that the proportion of unsaturated fatty acids must be substantially increased to make a spreadable milk fat-based product. This can be achieved outside of the butter regulations by adding liquid vegetable oil to the butter or cream to reduce solid fat content. This is the basis of dairy spreads and there are now a number of different types of dairy spreads available with improved spreading properties and a range of fat contents (Table 9.1).

The traditional dairy blend such as the Swedish product Bregott, launched in 1976, was prepared by injecting vegetable oil, usually canola or rapeseed, into the cream prior to churning in a continuous butter maker. Because the blend of butter and oil is softer and more spreadable at refrigeration temperatures than butter, it is necessary to churn the cream and oil mixture at a lower temperature, 5°C (41°F), and maintain this temperature throughout the process. Obviously it is important that the vegetable oil selected does not solidify at this temperature. The final product typically has a fat content similar to butter but with 15% to 25% of the milk fat being replaced by vegetable oil. Alternatively, injection of the vegetable oil into the butter rather than the cream has the advantage of reducing vegetable oil loss into the buttermilk, and the high-energy-cost/low temperature conditions for churning cream/vegetable oil blends are not required. Products prepared using butter (or cream) and vegetable oils may not be labeled as butter (Table 9.1).

Spreads Technology

The concentration of vegetable oil in traditional dairy spreads is limited because as butter is heated it displays a melting curve of solid fat content vs. temperature that is sigmoidal in shape, with a steep reduction in solid fat between 10°C (50°F) and 20°C (68°F). The addition of high amounts of vegetable oil results in a product with unacceptably low solid fat content, showing poor body and oiling off at room temperature (20°C; 68°F) (Figure 9.6). However, dairy spreads with higher proportions of vegetable oil and lower solid fat contents can be produced by adopting some of the technology and formulation aspects from the margarine industry.

Production of spreads with good plastic properties at refrigerator and room temperatures is achieved with the use of scraped-surface heat exchangers. A scraped-surface heat exchanger is typically a tubular heat exchanger cooled by a liquid refrigerant (for example, ammonia) with scraper blades mounted on a central shaft that rotates continuously, removing crystallized fat from the inner tube surface to promote rapid cooling and crystal nucleation in a short residence time (seconds). Scraped-surface heat exchanger units usually are employed in combination with crystallizer units, which hold a larger volume and have a longer residence time (several minutes). These units work the product between a series of metal pins fixed within the crystallizer tube and others mounted on a rotating central shaft, shearing the product, preventing formation of large crystal networks, and dispersing moisture droplets. The majority of crystallization of the fat takes place in the crystallizer unit(s) and the blend may then be transferred to resting tubes to continue crystallization, although with less shear, before packing.

Most manufacturers of spreads produce a range of products of differing fat contents and compositions to maximize their market. For example Dairy Crest, a major British manufacturer of butter and spreads, includes products within their portfolio of spreadable fat products with fat contents that range from

72% with a significant proportion of milk fat present (Clover) to as low as 19% (St. Ivel Gold range) and containing dairy ingredients but no milk fat. Clover (72% fat) is a mixed fat product that is produced using churn technology. It is comprised of vegetable oils, buttermilk (29%), water, skim milk, cream, salt, emulsifier, flavorings, vitamins A and D, and color. Approximately 50% of milk fat in Clover is replaced by vegetable oil, which necessitates the inclusion of some form of hard fat to provide a solid base that improves product body and reduces oiling off at room temperature. Clover has a slightly higher moisture content than butter, 20% compared to less than 16% for butter, and to aid dispersal of the moisture an emulsifier is added to the vegetable oil/buttermilk mixture.

In contrast, St Ivel Gold Extra Light plus Omega 3 (19% fat) is an example of the new generation of very-low-fat spreads designed to appeal to health conscious consumers. Its low fat content of just 19% fat attracts calorie-aware consumers, yet by enriching the spread with long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) C20:5, and docosahexaenoic acid (DHA) C22:6, it may also have a serious health role to help consumers increase their daily intake of these essential fatty acids, as recommended by various medical and health bodies. The aqueous phase in low-fat spreads is very important. In the St. Ivel product the aqueous phase is based on skim milk, buttermilk, and modified starch, and it is likely that interactions between the starch and protein ingredients in the blend are responsible for emulsion stability and viscosity. Other products have employed blends of maltodextrin and gelatine to similar effect. The amount and nature of structuring agent in the aqueous phase is critical, not only because of stability of the emulsion and viscosity but also because of effects on organoleptic properties.

Milk fat fractions are valuable ingredients in dairy spreads of varying fat content as an alternative to whole butter. The fractions may be emulsified into the cream for churning or processed using scraped-surface equipment. Consideration must be given to the solid fat contents necessary to deliver plastic properties over the functional temperature range from 5°C to 20°C (41°F to 68°F); hence, selection of fractions becomes critical.

There is little information in the open literature about the manufacture of very-low-fat products and what combination of technology and ingredients are needed to achieve desirable texture, stability, and mouth feel. Both water-in-oil type and oil-in-water low-fat spreads are available. Low- and very-low-fat dairy spreads compete with the margarine-type range of products rather than with butter or higher fat dairy spreads; hence dairy elements within such products have more to do with flavor and dairy image and less with the functional fat component. However, inclusion of dairy emulsifiers and flavorings such as milk protein introduces their own problems in the manufacturing process of spreads. Milk proteins favor oil-in-water emulsions, encourage formation of larger water droplets, and reduce the stability of water-in-oil products under such conditions as are applied during the latter stages of processing and tub filling (Moran 1994).

Following the introduction of worldwide regulations requiring the labeling of the trans fatty acid content in foods on health grounds, there is a drive by manufacturers of processed foods to reduce or eliminate industrial trans fatty acids from their products. This has led to replacement of the blend of hydrogenated and semi-hydrogenated vegetable oils that helped ensure plasticity in many of the spreadable fat products with ingredients containing no, or only trace amounts, of trans fatty acids.

One option is to blend liquid vegetable oils and butter with natural hard fats in proportions that vary with the fatty acid composition of winter (hard) and summer (soft) milk fat to ensure the final product has sufficient solid fat content for good body at

higher temperatures and yet continues to spread easily at refrigerator temperatures. More recently there has been considerable research into structured lipids to allow production of tailor-made fats with desired nutritional, chemical, and physical properties (Osborn and Akoh 2002). Generally, structured lipids are triacylglycerols that have been modified to incorporate new fatty acids or to change the position (distribution) of existing fatty acids along the glycerol backbone by chemically or enzymatically catalyzed reactions or genetic engineering. Inclusion of structured lipids in mixed-fat spreads is a novel approach to achieve products with good spreading properties and a relatively high content of milk fat or butter, but a low trans fatty acid content.

Concentrated Forms of Butter

Codex Alimentarius (Codex Stan 280–1973, revised 1999, amended 2006) describes the composition and quality standards for concentrated milk fat products: anhydrous milk fat (AMF), milk fat, anhydrous butter oil, butter oil, and ghee. These products are defined as fatty products derived exclusively from milk and/or products obtained from milk by means of processes which result in almost total removal of water and non-fat solids. Ghee is further defined as having an especially developed flavor and physical structure. The composition of these milk fat products is shown in Table 9.3.

Concentrated milk fat products are primarily used in recombined dairy products, confectionery, and bakery products. As ingredients they are less bulky than butter, have a long shelf-life, have a desirable milk fat flavor, and may be fractionated, blended, or similarly modified to enhance particular desirable characteristics and ensure consistency of properties. With the exception of ghee, the concentrated forms of milk fat are processed in a very similar fashion to produce light-colored, mild-flavored products of almost identical composition and with a wide range of possible product applications. Ghee, on the other hand, despite being almost identical in chemical composition to the other concentrated milk fat products, is subjected to heat treatments designed to induce marked changes to color, texture, and flavor, and is usually considered as a separate product with more traditional indigenous applications. Hence, AMF manufacture, as typical of the other concentrated milk fat products, is compared to the manufacture of ghee in this chapter.

Manufacture of Anhydrous Milk Fat

Anhydrous milk fat (AMF) may be manufactured from fresh cream or from butter (sweet cream, lactic, salted/unsalted) (Figure 9.7). The method of manufacture of AMF depends on the composition and structure of the source material. Regardless of the source, however, it is important that no chemical or

Table 9.3. Essential composition, contaminants, and quality factors of milk fat products.

	Anhydrous milk fat/ anhydrous butter oil	Milk fat	Butter oil	Ghee
% Minimum milk fat	99.8	99.6	99.6	99.6
% Maximum water	0.1	—	—	—
Copper maximum (mg/kg)	0.05	0.05	0.05	0.05
Iron maximum (mg/kg)	0.2	0.2	0.2	0.2
Maximum free fatty acids (% m/m as oleic acid) (V)	0.3	0.4	0.4	0.4
Maximum peroxide value (meq O_2/kg fat) (V)	0.3	0.6	0.6	0.6
Taste and odor (V)	Acceptable for market requirements after heating a sample to 40°C to 45°C			
Texture (V)	Smooth and fine granules to liquid, depending on temperature			

V, voluntary. Codex Standard 280–1973, revision 1999, amendment 2006

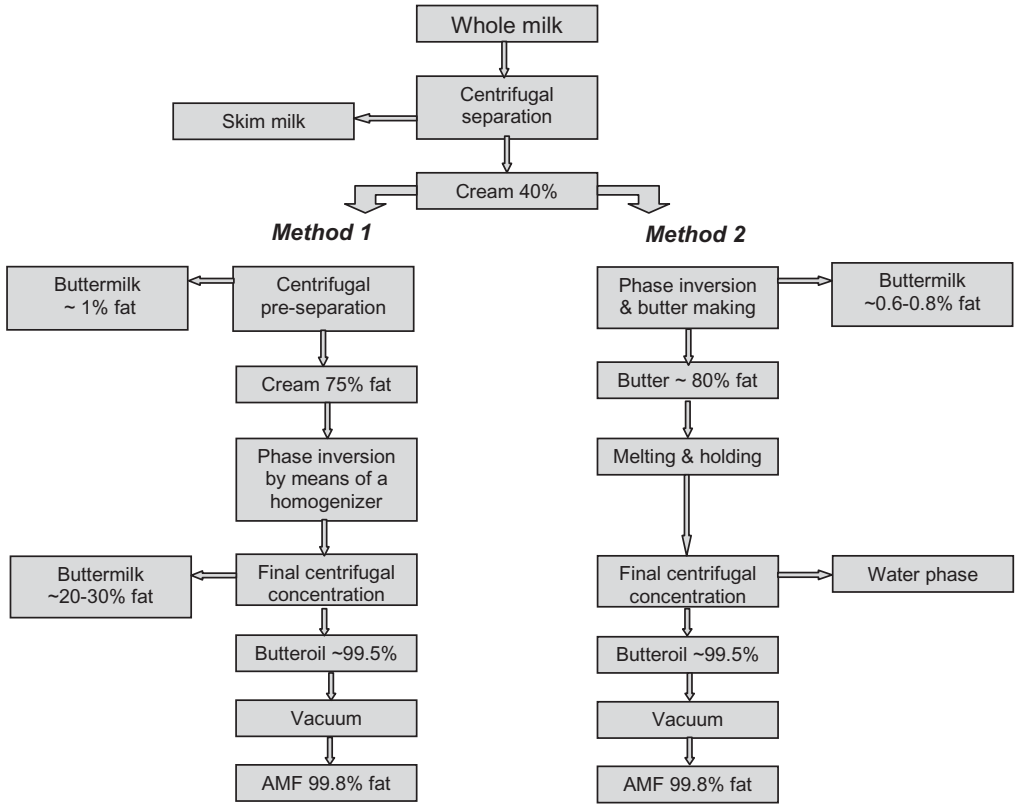

Figure 9.7. Anhydrous milk fat production from cream (method 1) and butter (method 2). Tetrapak Dairy Processing Handbook, UK.

microbiological deterioration of the original material has occurred to avoid transfer of off flavors into the concentrated product.

Cream is the starting point for AMF production from either source, approximately 75% to 80% fat if cream rather than butter is the starting material, or cream with a fat content of approximately 40% for butter manufacture. Usually the separated cream is pasteurized at a minimum of 85°C (185°F) to destroy bacteria and inactivate milk lipolytic enzymes. This prevents formation of free fatty acids (FFA) in the product, which are associated with off flavors. When using cream as the starting material, a second centrifugal concentration process is applied to obtain cream of 75% to 80% fat. The higher-fat cream ensures that effective phase inversion can be readily achieved at the next stage.

Phase inversion converts the oil-in-water cream emulsion into a water-in-oil emulsion, and although different types of devices are available, they all involve forcing the cream under pressure through narrow passages or orifices, as found in homogenizers (Figure 9.8). This process ruptures the fat globules in the absence of air, producing free fat and resulting in a water-in-oil emulsion. The product (99.5% fat) is then passed into a vacuum dryer where the moisture content of the oil is reduced to below 0.5%. This is achieved by first heating the oil to approximately 95°C to 98°C (203°F to 208.4°F) before drying it under vacuum, removing dissolved oxygen at the same time (Figure 9.9).

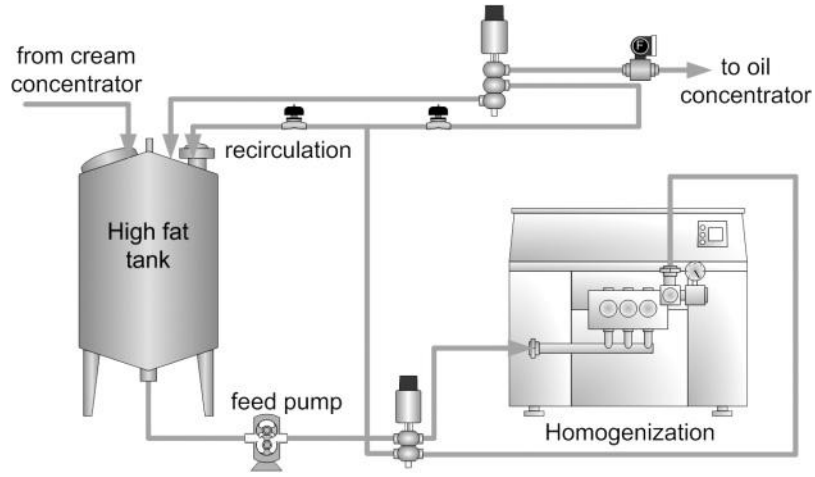

Figure 9.8. Phase inversion of cream for anhydrous milk fat production. GEA Westfalia Separator, Germany.

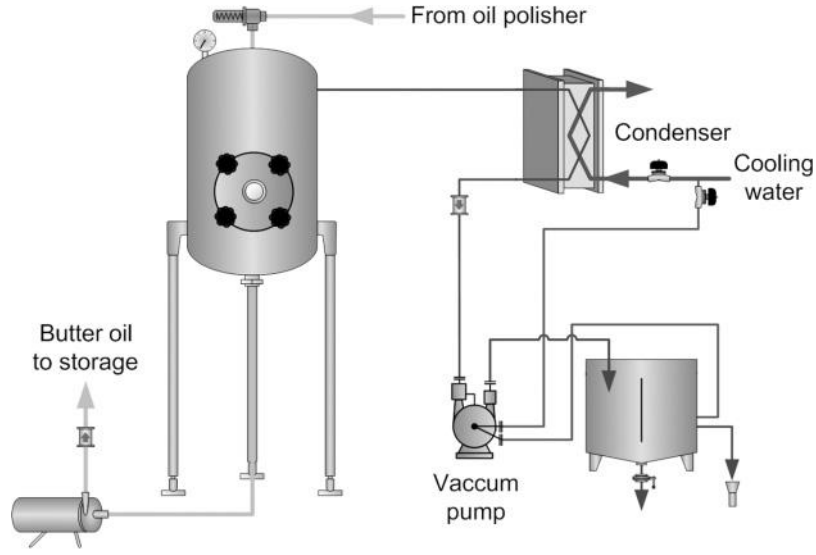

Figure 9.9. Vacuum drying of anhydrous milk fat. GEA Westfalia Separator, Germany.

The oil is then is cooled to 40°C (104°F) for packing. Typically AMF is packed in 1- to 20-liter containers for households and restaurants and 200-liter drums for industrial use. Normally an inert gas, nitrogen, is first injected into the container, where it sinks to the bottom. When the AMF is pumped in it replaces the nitrogen at the bottom of the container (AMF is heavier) and the nitrogen gas creates an oxygen-free layer on the surface of the oil. AMF may be stored at ambient temperatures for several months.

When AMF is prepared from butter (see previous butter manufacture section), unsalted butter is usually used and this is first melted by indirect means (plate heat exchanger) to 60°C to 70°C (140°F to 158°F). If direct heating by steam injection is used,

this creates an emulsion with small air bubbles as the dispersed phase, which is difficult to separate. After heating, the oil is held at the temperature for a short time to allow the proteins to aggregate before being pumped into the concentrator. Several separators may be operated in series to concentrate the butter oil and remove the separated sedimented solids. After concentration, the oil is heated to 90°C to 95°C (194°F to 203°F) and further dried in a vacuum dryer before packaging.

Refining

AMF may be refined for various purposes by polishing, neutralization, fractionation, and plasticizing.

Polishing

Polishing produces a clear, shiny (or bright) product. It entails the addition of water (20% to 30%) to the oil leaving the final concentrator, a short holding period, and then separation out of the water with removal of water-soluble solids, mainly protein (desludging).

Neutralization

Free fatty acids (FFA) contribute a distinctive flavor to dairy products but their content must be controlled to prevent adverse flavor development during storage. Neutralization of the FFA in AMF may be carried out by mixing dilute sodium hydroxide (8% to 10% weight/weight depending on FFA content) into the oil. After holding for a short period, hot water is added (20% to 30%) and the saponified FFA fraction is separated out in a centrifugal separator along with the aqueous phase. This process is not permitted by some countries.

Fractionation

The physical properties of milk fat make it unsuitable for many applications, especially some bakery and confectionery products in which a harder fat is required. Fractionation of milk fat to produce high- and low-melting fractions has addressed this problem to some extent. Unlike other processes employed by the edible oil industry to modify the physical properties of fats and oils, such as interesterification or hydrogenation, fractionation has minimal effect on the valuable milk fat flavor.

Fractionation of milk fat is normally carried out by dry fractionation only, that is, fractionation from the melt, without additives such as solvents or detergents. The molten AMF is slowly crystallized under controlled conditions to the desired temperature to crystallize out milk fat stearin (hard) and olein (soft) fractions. The crystallized fraction is harvested using special filters as the specific temperature is reached. The filtrate is then cooled to a lower temperature to crystallize another fraction, which is harvested, and the process continues. Fractionation of milk fat typically starts with a milk fat with a softening point (milk fat melts over a wide temperature range) of 33°C (91.4°F) and produces a hard fraction with a softening point around 43°C (109.4°F) and a soft fraction with a softening point around 21°C (69.8°F); a second soft fraction with a softening point below 10°C (50°F) also may be collected (Burgess 2001). The melting properties of the milk fat fractions differ depending on how they are obtained, that is, cooling rate, agitation, and number of steps employed in the fractionation process (Kaylegian 1999).

The selectivity of crystallization and efficiency of separation is lower in the dry crystallization process than when solvent is added. Triacylglycerols have a tendency to form solid solutions, which greatly reduces the efficiency of separation; hence, crystallization conditions for milk fat, with its high content of triacylglycerols, must be carefully managed to minimize their formation. Nucleation is the first step, and it occurs when the melted milk fat becomes supercooled, i.e.,

the temperature of the molten fat is much less than the thermodynamic equilibrium temperature. Slow, steady crystal growth is encouraged, with careful temperature control and minimal agitation to increase selectivity and avoid secondary nucleation, which would lead to an increase in small crystals and high viscosity, thus retarding crystal growth. A slow rate of cooling results in regular milk fat crystals that are easily filtered.

The semi-solid milk fat slurry is separated by vacuum or pressure filtration but entrainment of liquid fat within the crystal fraction decreases filtration quality and impairs the properties of the fraction. Processing factors also affect the efficiency of this part of the process, and these have been investigated by Vanhoutte et al. (2002, 2003). They reported that longer crystallizer residence times induced lower crystal growth and led to longer filtration times and lower oil entrapment, although yield was not affected. Higher agitation rates decreased filtration quality and increased yield of the high-melting fraction. Processing parameters such as fractionation temperature, crystallizer residence time, and agitation rate can be manipulated to produce fractions with different melting properties.

The membrane press filter is the preferred filtration technique in dry fractionation. Standard membrane press filters operate up to maximum pressures of 400 to 800 kPa and provide better separation than vacuum filters. The general principle of membrane filters is that a membrane is used to press the crystal slurry against (for example) a flat plate arrangement. The crystal slurry is held in a pouch between the membrane and plate and is pumped into the pouch under pressure. This squeezes out the majority of the liquid soft fraction and further pressure is then applied to squeeze out most of the remaining oil. The plates used to filter milk fat fractions are flexible membranes with a dimpled surface for easy drainage, unlike the rigid plate system used in the edible oil industry. Pressures up to 3,000 kPa are routinely used to inflate the flexible membranes and squeeze out the residual oil fraction. The high pressure used in dry fractionation of milk fat helps ensure a high yield from the process.

Plasticizing

If the anhydrous milk fat product is to be melted prior to use, then the temperature treatment pre- or post-packing has no effect on the functionality of the product. If, however, the milk fat product is to be used in a solid or semi-solid state, for example, incorporated into bakery goods, then the way in which it is worked and cooled (plasticized) markedly affects its functionality. Texturization of hard milk fat fractions for inclusion in pastries and croissants is achieved by physically reducing the size and increasing the number of milk fat crystals using high-pressure scraped-surface heat exchangers in combination with pin workers and setting (resting) tubes.

Agitation continues during crystallization to ensure the numerous small crystals formed remain discrete and independent. The texturized product is firmer with increased plasticity and the combination of cooling and working units can be adjusted to produce a range of products. At one extreme, texturized cake-making products, which soften rapidly when worked, and with good creaming and aeration properties, can be produced, while at the other extreme, products for pastries that can be rolled and worked with little appreciable softening or brittleness may be prepared. Emulsifiers such as mono- or di-glycerides also may be added to texturized milk fat fractions blended specifically for cake-making to further improve functionality.

Manufacture of Ghee

Ghee is similar in composition to AMF (Table 9.3), but is produced by a high-temperature process that confers a characteristic flavor. There are many indigenous

variations of ghee produced throughout Asia, the Middle East, and Africa, reflecting local preferences for the product (Sserunjogi et al. 1998). Ghee is primarily used for domestic culinary purposes, but it may be incorporated into confectionery products, used to garnish food, or fed to children for therapeutic purposes.

Ghee is typically prepared by heating cream or butter (from cows, buffaloes, camels, goats, or sheep) to a sufficiently high temperature at which it is held until most of the moisture has evaporated. The high temperature produces carbonyls (aldehydes and ketones), lactones, and FFA, which confer a characteristic flavor (varies between locales) while improving shelf life by destroying bacteria, inactivating enzymes, and forming reducing compounds (Sserunjogi et al. 1998). Over-heating, however, may lead to charring of milk solids-not-fat, discoloration, and even loss of desirable volatile flavor compounds.

Typically, in larger scale commercial production of ghee, fresh or cultured cream is heated to 115°C (239°F) in stainless-steel steam-jacketed vessels with continuous agitation until caramelization produces a golden brown color. When butter is the starting material, it is usually first heated to 60°C (140°F) and then transferred as a liquid into the steam-jacketed stainless-steel ghee boiler, where it is heated with continuous agitation to 90°C (194°F). Heating to a higher temperature may be carried out to develop a desirable color and flavor in the final product. The use of ripened milk, cream, or butter has been shown to produce an enhanced flavor in the ghee compared to the use of uncultured raw materials, and this is thought to be due to transfer of flavor metabolites such as free fatty acids and carbonyls from the aqueous phase of the original cultured source (Sserunjogi et al. 1998).

Ghee is generally packed into metal cans with a lacquered inner surface, although laminated pouches have also been used as a cheaper alternative. The shelf life of ghee is affected by its degree of unsaturation, but may be extended by storing a low-moisture product in opaque containers at a low temperature to reduce autoxidation. A good grainy texture due to the formation of large milk fat crystals is an important quality attribute of ghee; this can be encouraged by seeding liquid ghee with a small quantity of ghee grains. Ghee color and flavor are source- and process-dependant and tend to be determined by regional consumer preference.

Application of Milk Fat in Products

Milk fat plays an important role in dairy products and recombined dairy products such as cream, butter, ice cream, cheese, and concentrated milks, but it is also a valuable ingredient in a wide range of non-dairy products, primarily in the spreads, bakery, and confectionery sectors of the food industry (Table 9.4). As mentioned earlier, milk fat confers a unique flavor but its physical characteristics, which may be modified by fractionation and blending, also influence the texture, viscosity, firmness, and aeration ability of food products.

The production of recombined dairy products is widely used in areas that cannot sustain a local milk production industry. The main ingredients required for such products are non-fat milk solids, such as skimmed milk powder, and a concentrated fat source—AMF. These two ingredients may be blended together in the correct proportions with a variety of other ingredients to produce a range of recombined dairy products. Care must be taken to add the additional ingredients into the correct phase (for example, emulsifiers to the fat phase and salt to the aqueous phase).

For all recombined products it is important to use high-quality AMF or milk fat fractions that have been prepared, packaged, and stored to minimize oxidation and the development of flavor defects. Consideration also

Table 9.4. Application of milk fat ingredients in food products.

Application	Functional properties required	Milk fat
Laminated pastries such as Danishes, croissants, and puff pastry	Create barrier between layers of pastry. Good plasticity and resistance to work softening or melting during working. Flavor.	Traditionally butter was used. High-melting fractions (HMF) may be blended with AMF and then plasticized.
Cakes	Good aeration properties during creaming. Flavor.	Blends of medium- (MMF) and low-melting fractions (LMF) plasticized.
Biscuits and short pastry	Shortening ability achieved by coating flour particles with layer of fat to interrupt gluten network. Plasticity. Anti-bloom properties. Flavor (especially shortbread, Danish-type cookies).	Butter with solid fat content of 24% at 18°C. Blends of MMF and LMF plasticized.
Chocolate	Compatibility with cocoa butter to retain firmness, de-molding, snap, and shine characteristics. Anti-bloom properties. Flavor.	HMF or HMF blended with AMF.
Imitation chocolate coatings	Compatibility with lauric fats. Elasticity. Flavor.	LMF and MMF.
Dairy spreads	Mouth feel. Structure to hold liquid fat and moisture. Flavor.	Butter, AMF, LMF.
Recombined dairy products	Spreadability (recombined butter). Structure. Flavor. Whippability (ice cream).	AMF, AMF blended with LMF or MMF.

Adapted from Kaylegian, 1999.

must be given to achieving and maintaining emulsion stability within the recombined product because AMF and its fractions no longer contain valuable milk-emulsifying agents. Blending AMF with appropriate milk fat fractions can also improve the functional properties that are desired in the recombined product. For example, recombined butter tends to be firmer than traditional creamery butter, but blending a low-melting fraction with AMF helps improve its textural characteristics.

Chocolate

The main ingredients in milk chocolate are sugar, cocoa mass, cocoa butter, and milk powder (whole and skim). Milk fat may comprise up to 30% of the fat phase and it can have a critical impact on the texture and appearance of chocolate. During chocolate manufacture a careful temperature regime (tempering) is applied to ensure that the fat crystallizes in a stable form, beta 2 (form V); otherwise, the fat will transform to a more stable crystal form (beta 1 or form VI) during storage and change the glossy appearance of the chocolate to a dull, lighter one. This phenomenon is called "fat bloom" and is promoted by poor storage conditions such as cycling between warm and cool temperatures. Inclusion of milk fat inhibits bloom formation by stabilizing the beta 2 (form V) crystal form (Beckett 2000).

However, the addition of milk fat to cocoa butter softens the chocolate due to the presence of low-melting glycerides in milk fat and the fact that milk fat forms eutectic mixtures with cocoa butter, lowering the melting point of the fat mixture. The overall effect makes the chocolate mixture less functional and difficult to de-mold, and affects its "snap" characteristics. Because of milk fat's valuable flavor, mouth feel, and anti-bloom properties, there has been considerable research into the optimal levels and forms for inclusion of milk fat or milk fat fractions in chocolate and chocolate confectionery. High-melting milk fat fractions deliver good anti-bloom properties, and although there are conflicting reports regarding its effect on chocolate hardness in milk chocolate, the inclusion of a high-melting milk fat fraction in products such as truffles has been favorable (Sabariah et al. 1998).

Imitation chocolate, for example, coatings for ice cream, are based on vegetable fats of the lauric type. These lauric fats generally have poor compatibility with milk fat; however, it has been reported that the intermediate-melting fractions of milk fat can be mixed up to 40% inclusion with lauric fats with few detrimental effects on crystallization.

Baked Goods

The function of a fat in baked goods varies depending on the product. In laminated pastry (Danish, puff pastry, and croissant), the fat must be sufficiently plastic so that it can be rolled out between layers of dough without breaking, yet firm enough to avoid melting and absorption into the dough at the initial baking stages, thus providing a flaky texture in the final product. Although such products traditionally have been prepared with butter, seasonal variation in milk fat composition and an increase in mechanization of bakery processes have led to the development of blends of high-melting milk fat fractions and standard AMF specifically for these products (Kaylegian et al. 1993). Such milk fat blends have the advantage over margarines of retaining the valuable milk fat flavor and mouth feel.

In biscuits (cookies, shortbread), the role of the fat is to disrupt the formation of the gluten network by coating the flour particles with liquid fat, thus resulting in the characteristic "short" texture (Kaylegian 1999). Standard milk fat provides an optimum solid fat content at room temperature for biscuits, approximately 24%. Fat bloom can occur on

the surface of the biscuits, however, if the standard butter used has a higher solid fat content greater than this at room temperature (18°C to 20°C; 64.4°F to 68°F). The fat bloom is caused by fractional crystallization of large beta crystals of the higher-melting milk fat triacylglycerols, with an accompanying grayish white discoloration. The problem can be avoided by using a blend of low- and medium-melting milk fat fractions for this bakery application.

Cakes require fat to provide structure and lightness by helping entrap air during the creaming process, interrupting formation of the gluten network during baking, and providing emulsifying properties to retain liquid and keep the cake moist. Standard butter is not the best choice for cakes; it incorporates air well during creaming but its low solid : liquid ratio at room temperature means that the batter will break down easily. However, blends of medium and high-melting fractions of milk fat or blends of butter oil with margarines, especially with added emulsifier, can improve functionality.

Cheese

Processed cheese is made by blending different types of natural cheeses with emulsifiers and certain salts. Butter, butter oil, or AMF may be added to processed cheese to increase the fat content, either to produce a high-fat processed cheese or to compensate for reduction of fat in dry matter of the material after addition of emulsifying salts or other fat-free substances. Adding fat reduces the viscosity of the processed cheese and gives it a softer texture. There has been some research interest in the application of selected milk fat fractions in reduced-fat and low-fat cheese manufacture. Achieving the ideal physical attributes in the curd during manufacture is a problem with these cheeses, but the inclusion of lower melting milk fat fractions has been shown to modulate this (Rosenberg 2000).

Inclusion of AMF and low- and high-melting milk fat fractions in milk for mozzarella cheese manufacture also has been investigated (Rowney et al. 2003). Cheeses made with AMF contained predominately beta prime milk fat crystals; the hard-melting fraction contained predominately beta crystals, while the low-melting fraction contained more alpha milk fat crystals than the other cheeses. The implications here for cheese quality were that the cheese produced with the low-melting fraction had a lower solid : liquid milk fat ratio, resulting in more free oil in the cheese. Free oil formation in mozzarella cheese is a problem that can arise due to large seasonal variation in milk fat composition and melting point. The inclusion of a high-melting fraction with a higher solid : liquid milk fat ratio is an option to help manage the problem when seasonal soft milk fat is supplied to the factory.

Other Products

Satisfactory quality vanilla ice cream desserts (10% fat) have been made by substituting cream with AMF, a low-melting milk fat fraction, or a very-high-melting milk fat fraction. However, some differences between milk fat sources were observed during manufacture. The highest amount of solidified fat was found in the mix containing the very-high-melting milk fat fraction, while the mix containing the low-melting milk fat fraction had the lowest amount of solidified fat but the highest amount of adsorbed protein at the surface of the fat globules. The hardness of the ice cream was not affected by the fat source but the ice cream made using the very-high-melting milk fat fraction showed greatest resistance to melting, a useful property in hot countries (Abd El-Rahman et al. 1997).

Quality Assurance of Milk Fat Products and Spreads

The Commission Regulation (EC) 273/2008 lays down detailed rules for the application of Council Regulation (EC) No. 1255/1999 regarding methods for the analysis and qual-

ity evaluation (chemical, physical, microbiological, and sensory) of milk and milk products. A list of reference methods from the International Standards Organization (ISO) and International Dairy Federation (IDF) to evaluate milk and milk product quality are detailed (Table 9.5). Other analyses for which no reference method exists, for example, sensory evaluation of butter (Tables 9.6 and 9.7), are described in detail in the regulation (Regulation No. 273/2008). Compositional standards such as fat, solids-not-fat, and moisture levels are set down, as are tests and standards for quality parameters such as fat purity (using gas chromatography) or fat deterioration (by determining peroxide value and fat acidity levels). Determination of fat and moisture in butter and spreads is now often accomplished using infrared (IR) and near infrared (NIR) absorption techniques, which can respond rapidly to the turnaround needed with continuous production, product composition ranges, and physical form.

Off Flavor Development

Some aspects of quality are less straightforward to monitor than composition. Milk fat flavor comes from approximately 120 different compounds, of which the main flavor components are free fatty acids, lactones, and methyl ketones. The microbiological quality of the raw cream and thermal treatments applied during processing has a significant effect on flavor development (Chapter 12). Heating encourages formation of flavor precursors and promotes Maillard reactions and associated protein/carbonyl interactions. The high temperatures applied in the manufacture of ghee, for example, encourage development of a "cooked" flavor due to formation of methyl ketones, while some oxidation of the fat and lactone formation also contribute to the characteristic ghee flavor. The use of cultured products to manufacture ghee introduces another characteristic flavor compound, diacetyl, generated by the action of starter cultures used for lactic acid production.

In concentrated milk fat products flavor deterioration may occur via oxidation, lipolysis, or heat-induced breakdown of flavor precursors.

Oxidation and Lipolysis

Although cream for butter or AMF manufacture is used fresh, butter itself or AMF may be stored for some time before repackaging for market, refining, or incorporation into a product. Butter is often stored as 25-kg blocks for extended periods of time (four to nine months) in refrigerated or -20°C (-4°F) storage. It has been shown that butter stored at refrigerator temperatures exhibits a stale off flavor and increased levels of oxidation more rapidly than butter stored at -20°C (-4°F) (Krause et al. 2008). AMF is commonly held in 200-kg drums or 20-liter small metal containers at ambient temperatures for several months or at -20°C (-4°F) in plastic-lined cartons. High ambient temperatures (30°C; 86°F or greater) as occur in tropical countries reduce shelf life by causing more rapid deterioration of the milk fat and development of stale, oxidized flavor (Wilbey 1991).

Oxidation is the reaction between oxygen and the unsaturated fatty acids in the triacylglycerols. It may be catalyzed by light and metals such as copper and iron and the rate of oxidation increases with temperature. Oxidation is the main cause of flavor deterioration in AMF but it may be inhibited by reducing dissolved oxygen and headspace oxygen during manufacture and packing and eliminating contact with copper and iron. Oxidation may be assessed by measuring the peroxide value (PV), with a standard of less than 0.5 mEq oxygen/kg fat (Commission Regulation (EC) No. 273/2008) (Table 9.5). Compared with many fats, milk fat is relatively stable toward oxidation, although it has been reported that milk fat with a PV of 0.3 to 0.4 mEq oxygen/kg fat had an oxidized

Table 9.5. List of reference methods.

Product Regulation No. 1898/2005	Parameter	Limit	Reference Method
Unsalted butter	Fat	Minimum 82%	ISO 17189:2003/IDF 194:2003
	Water	Up to 16%	ISO 3727 1:2001/IDF 80:1:2001
	Solids-not-fat (SNF)	Up to 2%	ISO 3727 1:2001/IDF 80:1:2001
	Fat acidity	1.2 mmole/100 g of fat	ISO 1740:2004/IDF 6:2004
	Non-milk fat	Absent	Determination of milk fat purity by GC analysis of triacylglycerols is described in Annex XX of Commission Regulation (EC) 273:2008
	Tracers: Sterols		
	Vanillin		Quantitative HPLC method described in Annex VI of Commission Regulation (EC) 273:2008
	Ethyl ester of carotenoic acid		Quantitative spectrophotometric method described in Annex VII of Commission Regulation (EC) 273:2008
	Triacylglycerols of enthanic acid		Gas chromatographic (GC) method described in Annex V of Commission Regulation (EC) 273:2008
Salted butter	As above		
	SNF excluding salt	Up to 2%	ISO 3727 1:2001/IDF 80:1:2001
	Salt	Up to 2%	ISO 15648:2004/IDF 179:2004
Concentrated butter	Fat	Minimum 99.8%	IDF 24:1964
	Water and SNF	Up to 0.2%	ISO 5536:2002/IDF 23:2002 (moisture); IDF 24:1964 (SNF)
	Fat acidity	1.2 mmole/100 g fat	ISO 1740:2004/IDF 6:2004
	PV (maximum)	0.5 mEq oxygen/kg fat	ISO 3976:2006/IDF74:2006
	Non-milk fat	Absent	Determination of milk fat purity by GC analysis of triacylglycerols is described in Annex XX of Commission Regulation (EC) 273:2008
	Flavor	Clean	
	Smell	Absence of foreign odors	
	Other	Absence of neutralizing agents, antioxidants, and preservatives	
	Tracers	As for unsalted butter	

ISO, International Standards Organization; IDF, International Dairy Federation. Commission Regulation (EC) No. 273/2008

Table 9.6. Scoring of butter for sensory evaluation.

Parameter	Maximum	Description	Required
Appearance	5	Very good; equal dry	4
Consistency	5	Very good; well spreadable	4
Flavor/Aroma	5	Very good; finest pure aroma	4

Adapted from Commission Regulation (EC) 273:2008

Table 9.7. Examples of some butter defects.

Parameter	Description
Appearance	Free moisture; not uniformly colored; streaky; oil separation; open texture; foreign matter; moldy
Consistency	Brittle or crumbly; doughy and greasy; sticky; hard; soft
Flavour and Aroma	Without flavor; foreign flavor; stale; cheesy; acid; yeasty; cooked flavor; feed flavor; oversalted; malty; chemical flavor

Adapted from Commission Regulation (EC) 273:2008

flavor and was unacceptable for some uses. A drawback of the PV test is that it measures early oxidation intermediate products, hydroperoxides, which can break down during storage and thus give a low PV result.

Hydrolytic deterioration of milk fat with FFA formation is caused by the action of lipolytic enzymes secreted by bacteria or native to the milk on milk fat triacylglycerols. Microbial lipases are secreted by psychrotrophic bacteria such as pseudomonads, which favor low temperatures for growth. A low count, however, may be misleading because enzymes may have already been produced by the bacteria before numbers were reduced by heat treatment. Moisture is required for lipolysis, so minimizing levels in the product helps inhibit the reaction.

FFA content may be measured by titration with a weak alkali with test results typically expressed as "% oleic acid" (1 ml 0.1 M NaOH is equivalent to 0.0282 g oleic acid). Typical FFA levels in AMF from a large-scale modern factory may be 0.15 to 0.25% as oleic acid. Management of flavor quality of milk fat is critical in the management of overall product quality, and prevention of flavor deterioration must be uppermost during selection of the raw material, application of processing parameters, and handling and storage of the finished product.

Other Quality Aspects

Purity of the milk fat, that is freedom from adulteration with other non-milk fat sources, may be tested using gas chromatography (Commission Regulation (EC) No. 273/2008) or traditional chemical tests (Reichart-Meissl, Polenske, and Kirschner values) based on the content of water-soluble and water-insoluble fatty acids present (Fearon 2003). Milk fat fatty acid composition varies naturally with season and the cows' diet. Summer grazing of fresh grass and supplementation with oilseeds increases unsaturated fatty acids in milk fat, whereas feeding combinations of fish oil and a vegetable oil rich in linoleic acid has been shown to increase the content of conjugated linoleic acid (CLA) (AbuGhazaleh 2008).

The content of cholesterol in dairy and non-dairy products containing milk fat has frequently been criticized by health professionals. It is now possible to purchase a variety of cholesterol-reduced butters and spreads, and decholesterolization also has been applied to AMF. One method used in

Europe is the addition of a modified starch, beta-cyclodextrine, to the liquid AMF. The starch molecules surround the cholesterol and it can then be separated out by centrifugation. Decholesterolization on a commercial scale has had problems either with expense, complexity, or the removal of the valuable milk fat flavor. The addition of phytosterols (plant sterols) to non-dairy spreads to inhibit intestinal cholesterol absorption has resulted in the successful launch of several products but as yet has not been applied to dairy spreads or butters (Mortensen 2009).

Physical properties such as melting point and solid fat index or solid fat content are commonly used as specifications for fats and oils. Milk fat has a wide melting range, from -40°C to 40°C (-40°F to 104°F), but an estimate of final melting point is quite useful in characterizing the fat. Solid fat content of milk fat (or its fractions) over a range of temperatures is more valuable and may be measured by pulsed nuclear magnetic resonance (NMR) spectroscopy, which has largely replaced the solid fat index technique based on the more time consuming dilatometric method. It is important to recognize, however, that temperature history and tempering conditions have a significant effect on solid fat contents measured. Solid fat content alone is not sufficient to predict functionality in a product and test runs of the milk fat ingredient in a product are the best guide.

Note: Some of the information in this chapter was derived from Chapter 11, Butter and Spreads: Manufacture and Quality Assurance, published in *Dairy Processing and Quality Assurance* (Wiley-Blackwell, 2008).

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Chapter 10

Principles of Cheese Technology

Ramesh C. Chandan and Rohit Kapoor

Introduction

The term cheese belongs to a group of fermented milk foods. Essentially, cheese is a medium-moisture product (30% to 50% water) with an extended shelf life. The cheese making process leads to partial dehydration which, along with addition of salt and lowering of pH, confers a preservative effect and safety for consumption. The water activity (A_W) of a food is an indicator of its stability, safety, and shelf life. The A_W of cheese is around 0.87 to 0.98, as compared to nearly 1 for milk. The A_W is lowered by partial dehydration, which results from the removal of whey from curd, the addition of sodium chloride, and the generation of soluble, low molecular weight nitrogenous compounds during ripening (Fox, 2003b; Singh and Cadwallader, 2008). Other factors with preservative effects include lactic acid content (lower pH than milk) and antimicrobial metabolites generated by the culture activity, and use of modern protective packaging techniques. Accordingly, the main milk components of cheese, (proteins, fat, and minerals) are concentrated and safeguarded from rapid deterioration by spoilage microorganisms. Cheese is, therefore, a concentrated dairy food of high nutritional density. It provides consumers with portability, variety and novelty of flavors and textures, sound nutrition, and safety of use.

At present, more than 1,400 varieties of cheese are enumerated in the World Cheese Exchange Database, about 400 of which are the most recognized. In reality, fewer than 25 varieties are popular around the world. The large variety is essentially the result of historical, geographical, and environmental origin. The varieties owe their distinct flavor and textural attributes to the use of milk of various mammals, different ingredients, processing procedures, ripening conditions, and the final composition of the cheese. In addition, various shapes, sizes, and configurations, including shredded and sliced versions, provide versatility of novel applications. Consumers use these products as ingredients in popular dishes or as ready-to-eat snacks. They are designed to be consumed as a spread or as slices in sandwiches, and to function as a dip or topping on snacks. As an ingredient in food products, it is essential to understand cheese's performance as it relates to its variety, age, and cost factors.

Cheese making requires four basic raw materials: good-quality milk, a coagulating enzyme (rennet) or coagulating acids, culture, and salt. Cheese can be made from cream; whole milk; reduced-fat, low-fat, or nonfat milk; or from mixtures thereof. Some cheeses are made from whey, whey cream, or whey-milk mixtures. Furthermore, milk of sheep, goats, water buffaloes, and other milk-producing animals yields distinct color, flavor, and texture profiles.

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At the turn of the 20th century, developments in melting processes, involving natural cheese of various ages, gave birth to a line of processed cheese products with controlled flavor, texture, functionality, and extended shelf life. This chapter discusses the principles of cheese making. Basic processes related to various varieties of cheese are discussed in Chapter 11.

More than 300 varieties of cheese are marketed in the United States. In 2008, total natural cheese production was 9,934 million pounds (IDFA, 2009). The Italian cheeses totaled 4,158 million pounds, American cheeses totaled 4,071 million pounds, and other cheeses constituted 1,705 million pounds. The largest volume in the Italian cheese group was mozzarella cheese, which accounted for 3,239 million pounds. In the American cheese group, cheddar cheese topped the list at 3,149 million pounds. In the same year, process cheese foods and cold pack amounted to 2,191 million pounds.

Definitions

Because of the complexities associated with various types of cheese, an exact definition of cheese is not feasible. A simplified definition follows. Natural cheese (in contrast to process cheese) is made directly from milk, cream, buttermilk, or whey. One category of natural cheese is the fresh, unripened variety that is obtained from milk, whey, or cream by direct addition of a food-grade acid or a coagulating enzyme (rennet) and collecting the curd. Another type of non-ripened/fresh cheese may be produced by coagulating milk with a lactic-acid-producing culture, and separating the solid curd from the watery liquid called whey.

The second category of natural cheese includes cured or ripened varieties. In this case, the curd obtained after coagulation with rennet and/or lactic-acid-producing cultures, is pressed and held at a specified temperature and time to generate different profiles of color, aroma, flavor, and texture. These cheese characteristics have both qualitatively and quantitatively distinct profiles that set them apart from each other. Ripening processes use activity of bacteria, molds, and yeasts in the interior body of cheese or at the cheese surface throughout the ripening period. In general, a controlled regime of ripening temperature and humidity is necessary to facilitate the growth of ripening microorganisms.

Process cheeses and cheese analogs represent a significant part of cheese industry, which has broadened the application of cheeses in our diet and as an ingredient in food processing.

Classification of Natural Cheeses

A number of varieties of cheese evolved over a long period of time. Concomitant with the agricultural revolution, the origin of cheese is believed to be some 8,000 years ago in the Fertile Crescent between the Tigris and Euphrates rivers (now Iraq). Cheese making spread to Egypt, Greece, and later the Roman Empire. Cheese making know-how was further nurtured in feudal centers and in monasteries in which several modern day varieties were developed. Cheese makers in the mountains of Europe particularly specialized in discreet varieties. Cheese making eventually was imported by European immigrants to colonial locations in North and South America, where more innovations were introduced. Thus, cheese attained commercial significance in countries such as the United States, Canada, Brazil, Australia, and New Zealand.

Cheese has been classified based on various criteria. However, the criteria are insufficient to provide a thorough classification that includes all the types of cheeses. Cheese may be classified as follows:

1. Based on the manufacturing procedure and ripening process

a. Acid coagulation and no ripening
   - Coagulated with lactic acid generated by bacterial cultures: baker's cheese, cottage cheese, cream cheese, Neufchatel cheese
   - Coagulated with direct addition of acid to hot milk: ricotta cheese, chhana, paneer, queso blanco

b. Rennet (chymosin) coagulation and ripening
   - Cheddar
   - Colby and other stirred curd/granular varieties
   - Surface ripened: Brick, Limburger, Port du Salut, Bel Paese, Tilsit
   - Semi-hard cheeses with small eyes: Edam, Gouda
   - Other semi-hard cheeses: Monterey, Muenster
   - Cheese with larger eyes: Swiss, gruyere, Samso
   - Italian cheeses: Pasta Filata (Stretched Curd; Mozzarella, Provolone), Hard Cheese (Asiago, Fontina), Grating (Very Hard; Parmesan, Romano)
   - Mold-ripened: Blue, Roquefort, Stilton; surface mold ripened: Brie, Camembert

2. Based on whether the cheese is ripened and the type of ripening. Figure 10.1 shows the classification based on these criteria.
3. Based on moisture content, firmness, and ripening microorganisms. Figure 10.2 shows the classification based on these criteria.

Principles of Cheese Making

Recent publications on cheese science and technology contain detailed information on many varieties of cheese. The reader is referred to Fox et al. (2000) for cheese science in general, Robinson and Wilbey (1998) for automation in the cheese industry; Fox (2003a) for an overview of cheeses, Bachmann et al. (2003) for Swiss-type cheeses, Banks (2003) and Clarke and Agarwal (2007) for cheddar-type cheese, Bockelmann (2003) for smear-ripened cheeses, Bottazi (2003) for extra-hard Italian cheeses, Kapoor and Metzger (2008) for pasteurized process cheeses; Chandan (2003) and Clarke and Potter (2007) for cottage cheese and soft cheeses; Souza (2003) for surface-mold ripened cheese, and Aneja et al (2002) and Chandan (2007b) for cheeses made by direct acidification. Cheese production involves several steps common to most varieties. Certain modifications at certain steps lead to distinct varieties of cheese. A general process is shown in Figure 10.3.

The basic raw materials for cheese manufacture are milk, color (optional), starter (culture), rennet, and salt. Figure 10.4 illustrates the interaction of milk constituents and various ingredients used for making natural cheese.

Due to the large growth in cheese production within the last 50 years, there has been an immense development in the automation and mechanization of commercial cheese manufacture. Early reviews on automation in the cheese industry date back to the 1970s (Olson, 1969, 1975). Robinson and Wilbey (1998) and Bylund (2003) have described in detail the present mechanization involved at each step of commercial cheese making. This chapter mainly concentrates on the basic principles of cheese technology and attempts to highlight the present day commercial cheese making practices associated with each step. The key stages of cheese manufacture are presented below.

Quality and Selection of Milk for Cheese Making

Milk is the major cost factor, accounting for as much as 98% of the cost of many cheese varieties. Therefore, care and special

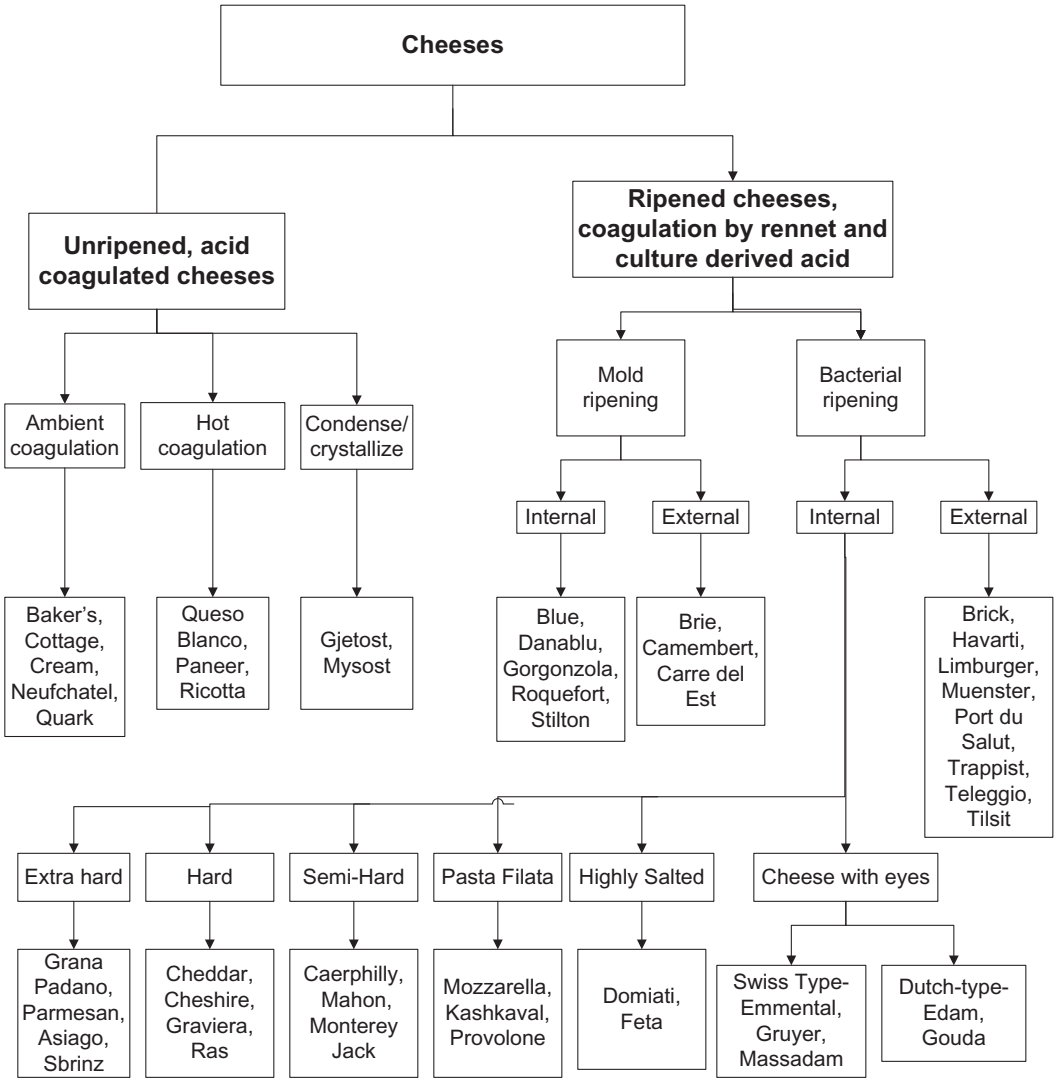

Figure 10.1. A classification of cheese based on whether the cheese is ripened and the type of ripening. Adapted from Fox (2003a).

attention is warranted in the milk procurement, handling, and processing phases of the cheese operation. The average content of 100 lbs of milk is 3.6 lbs of fat, 2.7 lbs of casein, 0.7 lb of whey proteins, 4.9 lbs of lactose, 0.7 lb of minerals, and 87.4 lbs of water (Chandan, 2007a). Thus, milk contains 12.6 lbs of total solids or dry matter, comprised of fat, protein, lactose, and minerals. The average fat in dry matter (FDM) of whole milk is $100 \times 3.6/12.6 = 28.6\%$. In cheddar cheese, the legal minimum for FDM is 50% and the moisture maximum is 39%. In fact, the Code of Federal Regulations (CFR, 2009) defines the composition of major cheese varieties in terms of fat and moisture content (Table 10.1). Cheese manufacturers must conform to these standards.

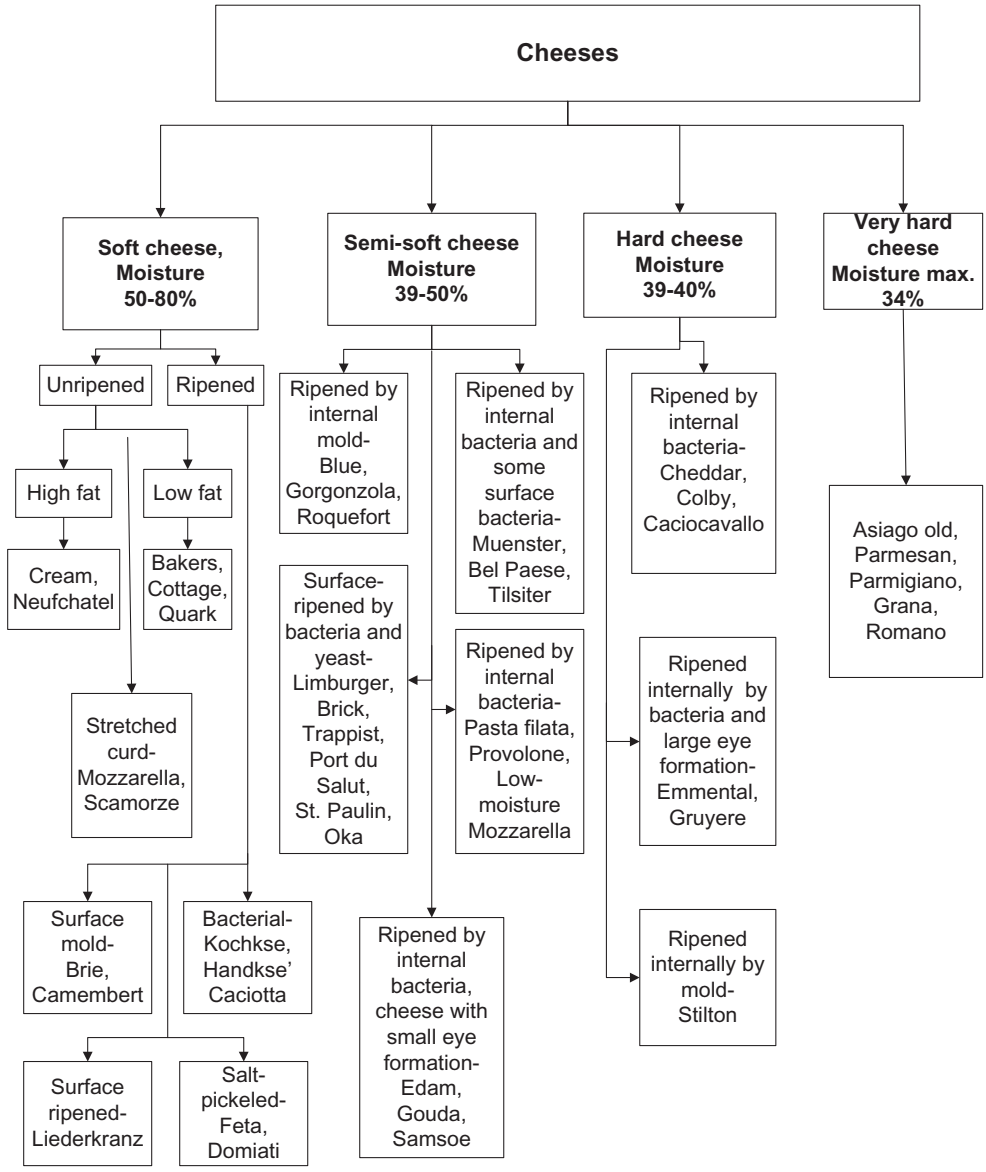

Figure 10.2. A classification of cheese based on moisture content, firmness, and ripening microorganisms. Adapted from Olson (1995 and 2003).

Many factors affect concentration of the components of milk, which in turn influences cheese yield. Recognition of such factors should help cheese plant operator to select incoming milk with quality factors to maximize cheese yield. In addition, the operator may react by modifying the processing parameters to achieve desirable quality and yield. Mastitis milk contains low casein, fat, and calcium, and must be avoided for cheese making. It also may contain antibiotics to treat mastitis, which in turn inhibit the growth

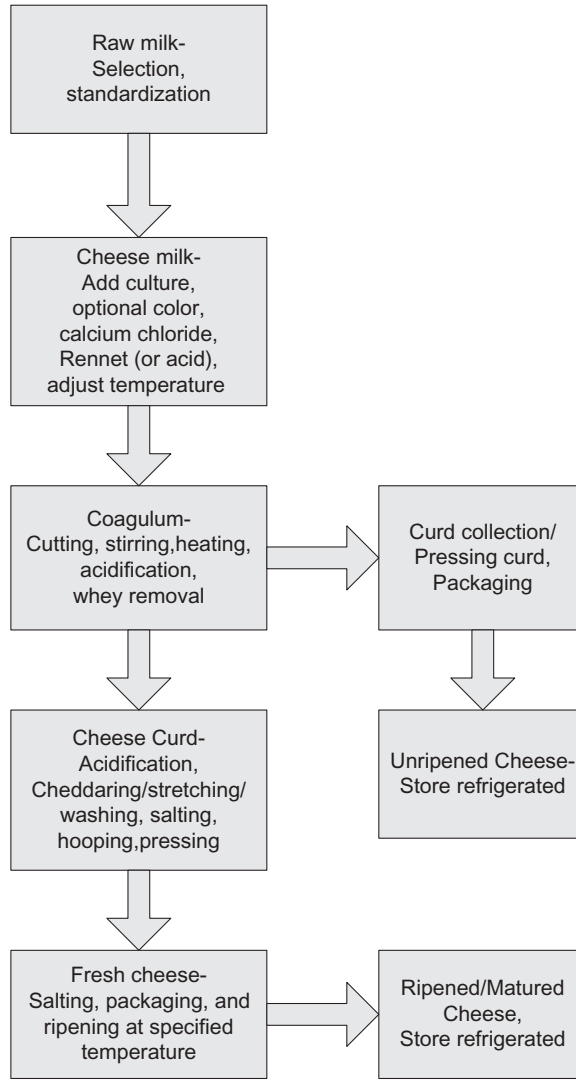

Figure 10.3. General steps for cheese manufacture.

of starter. Similarly, high somatic cells (exceeding 1 million/g) in incoming milk are deleterious for cheese yield. Therefore, cheese plants should screen milk for the presence of antibiotics and high somatic cells. Some genetic polymorphs of protein give better cheese quality and higher cheese yield. Accordingly, breeding cows for milk with such polymorphs is of interest to cheese plants. Seasonal variations in milk composition also affect cheese yield, but are beyond the control of cheese plants.

Cheese varieties are made from the milk of several mammals. Cows produce some 85% of world's milk supply, followed by water buffaloes at 11%, and sheep and goats at 2% each (Fox, 2003a). The composition of milk of the various species differs significantly (Chandan, 2007a). Making good quality cheese is challenging due to the dif-

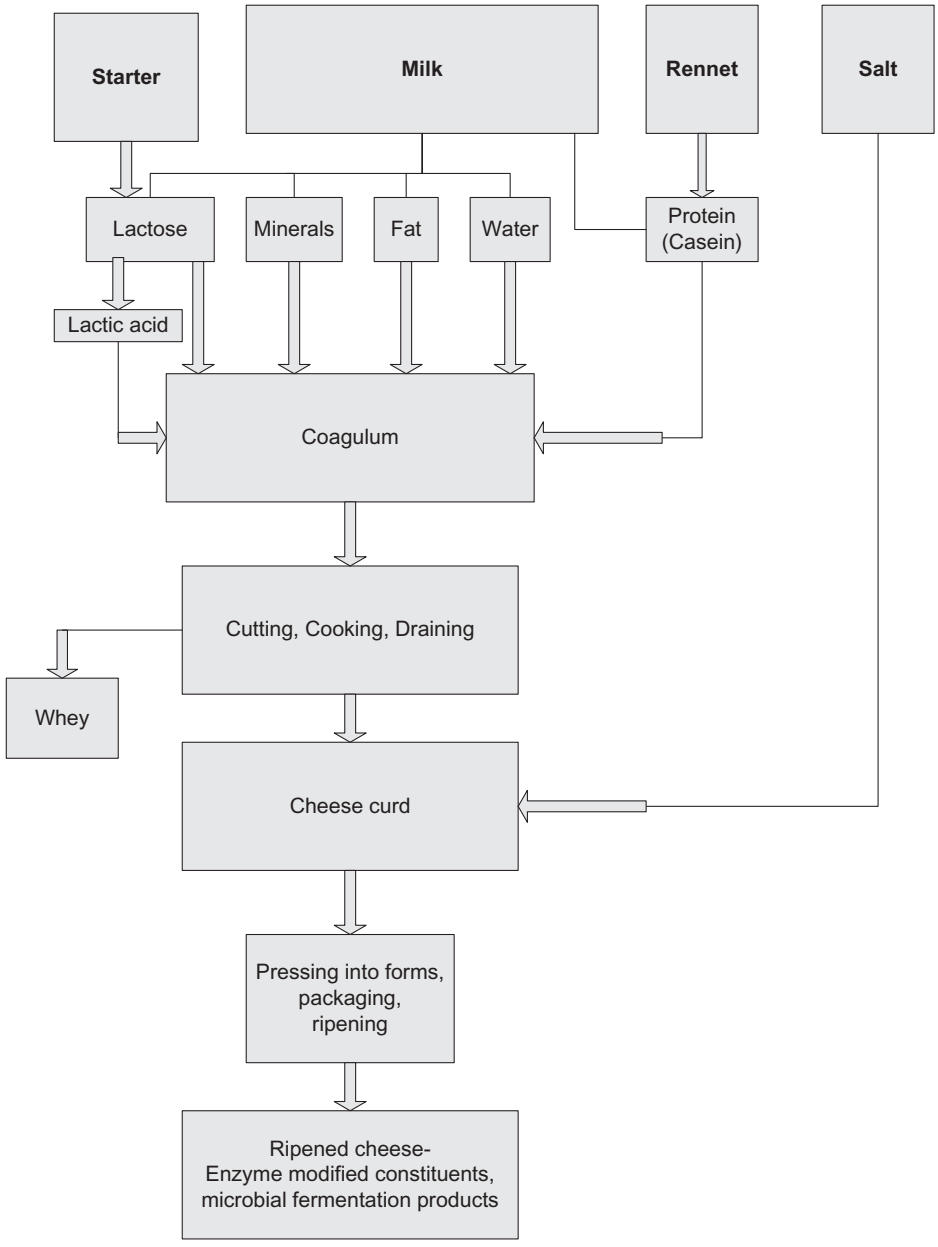

Figure 10.4. Relationships of milk constituents and ingredients in natural cheese.

ferent levels of casein fractions, whey proteins, and calcium content of the various species' milk. The world famous cheeses from buffalo milk include traditional mozzarella di buffalo. Sheep milk cheeses include Roquefort, feta, pecorino, Romano and manchego. Several goat's milk cheeses are specialty items and command premium prices. Goat's milk contains more α_{S1}-casein (one of the four casein fractions present in milk) than

Table 10.1. U.S. Food and Drug Administration compositional standards for natural cheeses.

Cheese	% Moisture, max.	% Fat in dry matter	Cheese	% Moisture, max.	% Fat in dry matter
Asiago, fresh	45	50	Monterey Jack	44	50
Asiago, medium	35	45	Mozzarella, Scamorza	52–60	45
Asiago, old	32	42	Mozzarella, low moisture	45–52	45
Blue	46	50	Mozzarella, part-skim	52–60	30–45
Brick	44	50	Mozzarella, part-skim, low moisture	45–52	30–45
Caciocavalle Sicilliano	40	42	Muenster	46	50
Camembert, soft ripened	—	50	Neufchatel	65	20–33*
Cheddar	39	50	Nuworld	46	50
Colby	40	50	Parmesan, reggiano	32	32
Cook, dry curd	80	—	Provolone, pasta filet	45	45
Cottage, dry curd	80	0.5[a]	Ricotta	80	11
Cottage, low fat	80	0.5–2*	Ricotta, skim milk	80	6–10
Cottage, creamed	80	4*	Romano	34	38
Cream	55	33*	Roquefort	45	50
Edam	45	40	Samsoe	41	45
Gammelost	52	—	Sap sago	38	—
Gorgonzola	42	50	Semi-soft	39–50	50
Gouda	45	46	Semi-soft, part skim	50	45–50
Granular, stirred curd	39	50	Skim milk cheese for manufacturing	50	—
Gruyere	39	45	Spiced	—	50
Hard cheeses	39	50	Spiced, part-skim	—	20–50
Hard, grating	34	32	Swiss, Emmental	41	43
Limburger	50	50			

*% fat in cheese *per se*

CFR: 21CFR133. Code of Federal Regulations, Title 21, Volume 2, Part 133. Food and Drug Administration, April 1, 2003. Pages 308–359

cow's milk, which makes it coagulate more easily. Because the fat in goat's milk contains smaller fat globules, the fat breakdown by lipases is accelerated. Fat recovery is higher and the curd possesses a smoother texture.

Milk for cottage cheese manufacture must conform to Grade A requirements (USDHHS, FDA 2003). In general, all milk supplied for cheese making should be of excellent microbiological quality. Due to bacterial growth, protein in milk may be degraded to soluble end products, thereby reducing the protein available for retention in cheese. Furthermore, microbial activity generates various off flavors in milk, which are carried into cheese and affect its quality. Most of the cheese made in the United States originates from pasteurized milk. It is generally accepted that cheese made from raw milk must be aged for at least 60 days to ensure safety from pathogenic infection.

Treatment and Standardization of Milk

Several approaches are used to remove the extraneous particles of raw milk. Raw milk may be passed through cloth filters or run through clarifiers to remove denser particles. If milk is run through a separator to remove part or all of the fat, heavier particles are collected in the bowl as sludge, while cream and skim milk streams flow out of the separator. Bactofugation is a high-speed centrifugal process that removes all bacterial cells and 95% of spores. It drastically reduces late gas defects in cheese caused by *Clostridium tyrobutyricum.* This process fractionates heavy casein particles, leucocytes, somatic cells, bacterial cells, and spores in 1% to 2% of the starting milk. For recovery, this fraction is sterilized by ultra-high-heat treatment and is subsequently added back to cheese milk. Some plants use microfiltration, which removes 99% of spores but can be used only for skim milk. Microfiltration membranes retain fat globules along with bacteria and spores.

The composition of a cheese variety determines whether milk should be adjusted for the protein:fat ratio. Predominantly, the casein:fat ratio of the starting milk determines the fat:protein ratio of cheese. A fat:casein ratio of 1.47 is generally considered optimum for cheddar cheese. Assuming a casein level at 2.2%, an optimal fat level of 3.2% in milk is desirable for making cheddar cheese. Different ratios are needed for other cheese varieties. For example, for mozzarella cheese, milk must be standardized to 1% to 3% fat, depending on the type of mozzarella. Part-skim mozzarella is made from 1% to 2% fat (0.37 : 0.75 fat : casein ratio), whereas regular mozzarella is made from 3% milk (fat:casein ratio of 1.11).

It is essential to standardize cheese milk to a specific fat content to achieve a specific fat:casein ratio in cheese. Various automated milk standardization instruments are being used in the cheese industry. Milk analyzers based on near infrared technology are among the techniques gaining popularity. These techniques improve production efficiency as well as the quality and consistency of the final product (Barbano and Lynch, 2006). Cheese milk can be standardized by one of the following methods discussed below.

Separator

Fat content in raw milk may be reduced and standardized by running milk through a separator, which partially or totally removes fat to achieve a desired level for a specific cheese. For example, partial removal of fat is required for part-skim mozzarella, and skim milk is needed for cottage cheese manufacture.

Addition of Skim Milk

The amount of skim milk to be added for reducing fat content of cheese milk is calculated as follows:

$$\text{Pounds of skim milk} = \text{Pounds of unstandardized milk} \times \frac{(\%\ \text{fat in unstandardized milk} - \%\ \text{fat in standardized milk})}{(\%\ \text{fat in standardized milk} - \%\ \text{fat in skim milk})}$$

A simplified version of this formula may be derived by assuming 0% fat in skim milk:

$$\text{Pounds of skim milk} = \text{Pounds of unstandardized milk} \times \frac{(\%\ \text{fat in unstandardized milk} - \%\ \text{fat in standardized milk})}{\%\ \text{fat in standardized milk}}$$

Addition of Cream

If the fat content of milk is too low (as in cream cheese manufacture), the amount of cream for standardization may be calculated:

$$\text{Pounds of cream} = \text{Pounds of milk} \times (\%\ \text{fat in standardized milk} - \%\ \text{fat in unstandardized milk}) / (\%\ \text{fat in cream} - \%\ \text{fat in standardized milk})$$

Addition of Low-heat Nonfat Dry Milk (NFDM)

For use in the above formula, the pounds of NFDM equivalent to skim milk may be calculated for standardizing cheese milk to any desirable fat content:

$$\text{Pounds of nonfat dry milk} = \text{Pounds of skim milk} \times 0.09$$

It is advisable to reconstitute NFDM with clean water to yield the calculated weight of skim milk. The reconstituted skim milk may then be used for standardization. Direct standardization with the addition of NFDM increases the total solids and alteration in moisture:solids ratio. Thus, the cheese yield/unit volume registers an increase. However, in most cheese varieties, modifications in the cheese making procedure may be necessary.

An alternate method of augmenting milk solids is the use of liquid condensed milk or liquid milk protein concentrate obtained as retentate from ultrafiltration of skim milk.

The adjustment of nonfat solids of cheese milk with NFDM is limited to a few percentage points of the weight of the milk; otherwise, the higher lactose content of the cheese would result in an abnormal fermentation pattern and poor cheese texture. Milk protein concentrate allows for better control of the lactose content of the cheese milk. However, the use of dry milk protein concentrate to standardize cheese milk is not allowed in the United States at present.

Preconcentrating the cheese milk to approximately 15% to 18% total solids via evaporation or ultrafiltration has become a common practice in the cheese industry to improve production efficiencies. Because evaporation of milk leads to an equal increase in all of the milk constituents, including lactose, cheese made with such milk requires changes in cheese-making protocols to ensure proper fermentation and ultimately the final cheese.

The preferred process is ultrafiltration, which selectively separates the milk into an enriched protein-fat fraction and water-lactose fraction. This enhances the cheese milk with the desirable solids such as protein and fat, and the lactose in the cheese milk tends to be at the same level (Mistry and Maubois, 2004). The use of ultrafiltration to produce whey-less hard cheeses such as cheddar also is gaining popularity in the cheese industry. The resulting "cheddar cheese for manufacture" (21CFR 133.114) is used as an ingredient in pasteurized process cheese manufacture.

Heat Treatment of Milk

Raw milk is better suited for certain cheese varieties. However, for public health and

safety reasons, most of the world's cheese is produced with pasteurized milk. Some plants use thermization treatment to prevent spoilage of stored raw milk over weekends; the technique involves heating raw milk at 63°C to 65°C (145°F to 149°F) for a few seconds. Nevertheless, milk still must be pasteurized prior to cheese making.

Pasteurization inactivates heat-labile enzymes and reduces bacterial load. Pasteurized milk must test phosphatase negative. Generally, in the United States, FDA regulations require pasteurization of milk at 71.7°C (161°F) for 15 seconds for cheeses consumed fresh. If milk is not pasteurized for cheese making, the cheese must be held for at least 60 days at 1.67°C (35°F) or higher prior to consumption. The equivalent heat treatment is 63°C (145°F) for 30 minutes. Heat treatment of milk at a higher temperature-time regime has deleterious effects on milk coagulation and the physical quality of cheese.

Additives in Cheese Milk.

Calcium chloride is added to milk at approximately the 0.02% level to accelerate coagulation and to improve cheese yield.

Cheese color is added to produce certain cheeses of consistent color throughout the year. One example is annatto dye, an oil extract of seeds of the *Bixa orellana* plant. Annatto contains two apocaroteinoid pigments, bixin and norbixin, that do not have vitamin A activity. The color is red to yellow and is pH dependent. The yellow coloration is more pronounced at higher pH and changes to a reddish hue at the normal pH of cheese. At a pH of under 4.8, it turns light pink and eventually colorless. Bleaching and pink coloration in cheese is due to oxidation of annatto caused by heat treatment, ultraviolet light, iron, copper, and chlorine. β-carotein, another colorant used in cheese, imparts too much yellow hue.

Bleaching agents are used in some cheeses made from cow's milk to simulate the white appearance of milk of water buffaloes, goats or sheep. Titanium dioxide and chlorophyll based-colorants are permitted in many countries.

Certain enzymes are used as ripening supplements. These are generally lipases and proteases developed from starter cultures, which are used to accelerate cheese ripening. Other enzyme preparations are derived from the buccal cavity of young goats and sheep, which consist of concentrates of esterases. When cow's milk is substituted for goat or sheep milk, these enzyme preparations simulate the development of traditional flavors of feta, Romano, and parmesan cheeses.

Starter

Milk is normal habitat of several lactic acid bacteria that may cause spontaneous souring under favorable conditions. Specially prepared lactic acid bacteria called starters are used for cheese production control (Broome et al., 2003; Powell, 2003; Hill, 2009). A starter consists of harmless microorganisms that impart desirable and predictable characteristics to a given variety of cheese upon culturing in milk and cheese curd. The starter bacteria are non-motile, Gram-positive, non-sporeforming, catalase, and nitrate negative, and are microaerophilic (prefer low oxygen concentration) in nature. They are generally inactivated at refrigeration temperature. Under microscope, the lactococci appear round/elliptical (cocci) shaped with diameter of approximately 1 μM. The lactobacilli are rod shaped, 1 μM in width and 2 to 3 μM length. Depending on the cheese, the starters contain mesophilic or thermophilic cultures. They are distinguished by their optimum growth temperature, which is approximately 30°C to 35°C (86°F to 95°F) for mesophilic cultures, and 39°C to 50°C (102°F to 122°F) for thermophilic cultures. As the starter culture grows, its metabolic end products lead to acidification of milk and curd. Furthermore, evolution of CO_2 can form holes (eyes) as in Swiss cheese. Production

Table 10.2. Attributes of typical mesophilic cultures used in cheese making.

Attribute	*Lactococcus lactis* ssp. *lactis*	*Lactococcus lactis* ssp. *cremoris*	*Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis*	*Leuconostoc lactis*	*Leuconostoc mesentroides ssp. cremoris*
Cell shape and configuration	Cocci, Pairs, short chains	Cocci, pairs, short/long chains	Cocci, pairs, short chains	Cocci, pairs, short/long chains	Cocci, pairs, chains
Catalase reaction	–	–	–	–	–
Growth temperature (°C)					
Optimum	28–31	22	28	20–25	20–25
Minimum	8–10	8–10	8–10	4–10	4–10
Maximum	40	37–39	40	37	37
Incubation temperature (°C)	21–30	22–30	22–28	22	22
Heat tolerance, 60°C/30 minutes	±	±	±	–	–
Lactic acid isomers	L(+)	L(+)	L(+)	D(–)	D(–)
% Lactic acid produced in milk	0.8–1.0	0.8–1.0	0.4–0.8	0.1–0.3	0.1–0.3
% Acetic acid produced in milk	–	–	–	0.2–0.4	0.2–0.4
Gas (CO_2) production	–	–	+	±	±
Proteolytic activity	+	+	+	±	±
Lipolytic activity	±	±	±	±	±
Citrate fermentation	–	–	+	+	+
Flavor/aroma compounds	+	+	+++	+++	+++
Mucopoly- saccharide production	±	±	±	–	–
Hydrogen peroxide production	+	+	+	±	±
Alcohol production	±	±	±	±	±
Salt tolerance (% maximum)	4–6.5	4.0	4–6.5	6.5	6.5

Adapted from Chandan and Shahani (1995)

of diacetyl imparts a buttery flavor in cream and cottage cheese. The salient features of various cultures contained in starters are shown in Tables 10.2 and 10.3.

Starter culture basically has two major functions. One is to produce acidity during cheese making, and the other is to aid in ripening of cheese. Acid development leads to milk coagulation in acid-coagulated cheeses, a key step in cheese making. In rennet-coagulated cheeses, acid development accelerates coagulation. Acidity (low pH) discourages the growth of pathogenic and spoilage bacteria, thereby imparting a food safety property to cheese. Acidity promotes contraction and removal of water from curd matrix (syneresis); thus, it is a tool for cheese moisture control. Furthermore, it is involved in cheese flavor and texture development. If acidity is too low, off flavors such bitterness, fruitiness, and rancid flavors develop in cheese. On the other hand, high acidity leads to a brittle texture and mottled cheese color. During ripening, starter culture produces factors for facilitating growth of non-starter organisms which collectively are responsible for desirable flavor and texture. They also furnish proteolytic and lipolytic enzymes involved in cheese ripening.

Secondary cultures (Rattray, 2003) are used for special functionality. *Propionibacterium freudenreichii* subsp. *shermanii* pro-

Table 10.3. Some characteristics of thermophilic strains used in cheese making.

Characteristic	*Streptococcus thermophilus*	*Lactobacillus delbrueckii* ssp. *bulgaricus*	*Lactobacillus delbrueckii* ssp. *lactis*	*Lactobacillus casei* ssp. *casei*
Cell shape and configuration	Spherical to ovoid, pairs to long chains	Rods with round ends, single/short chains, metachromatic granules	Rods, round ends, metachromatic granules	Rods with square ends, short/ long chains
Catalase reaction	–	–	–	–
Growth temperature (°C)				
Optimum	40–45	40–45	40–45	37
Minimum	20	22	22	15–20
Maximum	50	52	52	40–45
Incubation Temp. (°C)	40–45	42	40–45	37
Heat tolerance, 60°C/30 minutes	++	+	+	–
Lactic acid isomers	L(+)	D(–)	D(–)	L(+)
% Lactic acid produced in milk	0.7–0.8	1.5–4.0	1.5–3.0	1.2–1.5
% Acetic acid produced in milk	Trace	Trace	Trace	+
Gas (CO_2) production	–	–	–	–
Proteolytic activity	±	+	+	±
Lipolytic activity	±	±	±	±
Citrate fermentation	–	–	–	–
Flavor/aroma compounds	++	++	+	±
Mucopoly-saccharide production	±	++	–	±
Hydrogen peroxide production	±	+	+	+
Alcohol production	–	Trace	Trace	Trace
Salt tolerance (% maximum)	2.0	2.0	2.0	2.0

Adapted from Chandan and Shahani (1995)

duces CO_2, which generates large holes in the interior of cheese (eyes) in Swiss cheese varieties when under pressure. Surface smear-ripening organisms consisting of *Brevibacterium linens*, micrococci, and certain yeasts and molds produce distinct flavors and textures, while blue mold (*Penicillium roqueforti/glaucum*) develops a blue-veined appearance and characteristic flavor. A white mutant of *Penicillium roqueforti* is used in Nuworld cheese production, imparting a typical blue cheese flavor with white veins. Similarly, white mold (*Penicillium caseicolum/ candidum, camembertii*) gives a snow-white appearance and discreet flavor profile to Camembert and Brie cheeses.

Accordingly, the bacteria and fungi present in the starter leave an imprint on cheese flavor and texture. Other cultures, added along with starter cultures, are used as ripening adjuncts. They may be bacterial or yeast cultures, or non-growing attenuated cultures designed to furnish desirable enzymes. For instance, certain lactobacilli and pediococci are also used; they grow during cheese ripening and deliver ripening enzymes. Consequently, the basic character and individuality of a cheese are governed by the type, composition, growth, and metabolic attributes of the starter. Besides the genus and species of the organism, it is important to remember that starters may contain various

Table 10.4. Microbial composition of starters used in the manufacture of major cheese varieties. Two or more starters may be used in some cheeses.

Cheese varieties	Microorganism
Cheddar, colby, Gouda, Edam, Monterrey	*Lactococcus lactis* ssp. *lactis* *Lactococcus lactis* ssp. *cremoris*
Cream, Neufchatel, cottage	*Lactococcus lactis* ssp. *lactis* *Lactococcus lactis* ssp. *cremoris* *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* Leuconostoc mesentroides ssp. cremoris
Swiss, Emental, gruyere, Samso, fontina	*Leuconostoc lactis* ssp. *cremoris* *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* *Leuconostoc lactis* ssp. *cremoris* *Lactobacillus delbrueckii* ssp. *bulgaricus* *Lactobacillus delbrueckii* ssp. *lactis* *Lactobacillus casei* ssp. *casei* *Lactobacillus helveticus* *Streptococcus thermophilus* *Propionibacterium freudenreichii* *Propionibacterium shermanii*
Italian cheeses: Mozzarella, provolone, romano	*Lactococcus lactis* ssp. *lactis* *Streptococcus thermophilus* *Lactobacillus delbrueckii* ssp. *bulgaricus* *Lactobacillus delbrueckii* ssp. *lactis* *Lactobacillus helveticus*
Brick, Limburger, Muenster, Trappist, Port Salut, St. Paulin, Bel Paese, tilsit	*Streptococcus thermophilus* *Lactobacillus delbrueckii* ssp. *lactis*
Blue-veined cheeses: Roquefort, bleu, Stilton, Gorgonzola	*Lactococcus lactis* ssp. *lactis* *Lactococcus lactis* ssp. *cremoris* *Penicillium roqueforti*
Camembert, Brie	*Lactococcus lactis* ssp. *lactis* *Lactococcus lactis* ssp. *cremoris* *Streptococcus thermophilus* *Penicillium candidum* *Penicillium caseicolum* *Penicillium camemberti*

strains of the same organism. Table 10.4 shows the composition of various primary starters used for cheese making.

A potential problem in cheese plants is related to infection by bacteriophages; as parasites, they depend on host bacteria for their own life cycle. They are virus-like particles that destroy culture bacteria in 30 to 40 minutes of contact. A phage consists of a head and a tail. The tail section attaches itself to the bacterial cell wall, penetrates it, and injects its DNA into bacteria. The bacteria begin producing phage DNA and protein, which form fresh phages. Eventually, the bacterial cell explodes, releasing 50 to 100 new phages/bacterial cell. The new phages further invade more bacterial cells, lysing them in turn, and destroying culture activity. Their attack can result in significant economic losses. Phages require Ca^{+2} for their growth; thus, sequestering Ca^{+2} with phosphate in the growth medium inhibits phage proliferation. Because phages are strain-specific, rotating cultures is a practical way to evade their attack. Cheese plants may rotate as many as 10 cultures every day. Other measures for phage control include strict plant sanitation, use of phage-resistant media and strains, direct-to-vat cultures, and avoiding whey storage in the plant. Various types of starters are available for selection in a cheese plant.

Pure, Defined Single-strain Starters

Pure, defined single-strain starters are selected from mixed strains for specific characteristics such as phage resistance or optimum proteolytic profile. Advancement in starter production technology has resulted in single-strain starter concentrates which, by culture rotation techniques coupled with plant sanitation, have dominated their use in larger cheese plants. Defined single-strain cultures have the advantage of uniformity of acid production and flavor profile of cheese. They must be rotated to avoid phage infection.

Mixed Defined Strain

Mixed defined strain cultures are blends of single strain cultures. They must be rotated to control phage attack. They relatively yield uniformity of acid production and flavor profile.

Multi-strain Starters

Multi-strain starters are non-specific, undefined mixtures. Their advantage over single-strain starters is that acid development is likely to continue even in the presence of a phage that is capable of attacking only a single strain. However, it is known that ultimately a single strain may dominate at the expense of others. Mixed mesophilic cultures are still used in the cheese industry, but normally the thermophilic cultures are mixed single-strain types. The disadvantages of mixed cultures are a wide variation in the rate of acid development as well non-uniformity of cheese flavor from vat to vat.

Various starters are available from commercial culture suppliers. Concentrated cultures are available in liquid, frozen, or freeze-dried forms. Frozen cultures require expensive sub-zero transportation and storage. Freeze-dried culture concentrates are cheaper to transport and store. Some plants propagate culture to prepare their own bulk starter.

To prepare bulk starter for inoculation, the concentrate is transferred to a medium consisting of heat-treated milk, reconstituted NFDM, or a special whey-based phage-resistant phosphate medium in a bulk starter tank and allowed to grow under optimum conditions. It is then cooled and used at 0.5% to 1% inoculation level. Refinements in starter technology have led to production of single-strain starter concentrates (direct-to-vat set/inoculation), which require no sub-culturing, bulk setting, or activation. Their use requires simple dilution with pasteurized milk to furnish adequate active cells to initiate acid development in the cheese vat. Having a working relationship with the culture supplier helps to optimize their use and prevent phage attack, which could slow acid production or even kill the culture, leading to huge losses in a cheese plant. Culture suppliers have information on phage typing and methods to avoid phage attack by culture rotation.

The quantity of the starter is important for the rate of acid development, moisture retention, flavor, and texture of cheese. Higher rates of inoculation may lead to undesirable fast acid development. It is advisable to follow the recommendation of the culture supplier. The temperature of setting milk and cooking conditions reflect the optimum growth temperature and heat ruggedness of the culture involved.

Production of many cheeses depends on *Lactococcus lactis* subspecies *lactis* and *cremoris* for acidity development. These cultures belong to the mesophilic group. Their acid production is optimum at 30°C to 35°C (86°F to 95°F). However, acid production essentially stops at temperatures below 20°C (68°F) and above 39°C (102°F). The *cremoris* sub-specie is generally regarded as the best for optimum cheese flavor. The subspecie *lactis*, however, is a better acid developer. Therefore, the two are often blended in

cheese starters. Bio-variant specie *diactely-lactis*, also called *Lactococcus lactis* citrate+, produces CO_2 and a buttery flavor compound (diacetyl) from citrate, a normal constituent of milk. A weak acid producer, *Leuconostoc mesentroides* ssp. *cremoris*, also produces diacetyl and CO_2. The flavor compound (diacetyl) is essential in fresh cheese production. These are used in soft ripened, cheddar, most washed, and fresh cheese varieties.

Thermophilic starters are traditionally used in Swiss, gruyere, and some Italian cheeses such as mozzarella. In addition to lactic acid, these cultures characteristically produce acetaldehyde. Thermophilic starters consist of cultures capable of growth at temperatures from 39°C to 50°C (102°F to 122°F). Minimum growth is at 20°C (68°F), but they are partially inactivated at temperatures below 20°C (68°F). They can survive at 55°C (131°F). Thermophilic starters are blends of cocci and rods. The cocci consist of *Streptococcus thermophilus* (ST) and the rods are *Lactobacillus delbrueckii* subspecies *bulgaricus* (LB), *lactis* and *helveticus.* The traditional cultures of yogurt, ST and LB, grow together in a symbiotic relationship, which implies that together their rate of acid production is faster than that of either culture individually. The balance between rods and cocci can be controlled by temperature manipulation. Higher temperatures favor rods and lower temperatures stimulate cocci. Rods are more resistant to high acid conditions. It is useful to know how the temperature and salt level affect the growth of a starter because they are important tools in manipulating and controlling their effects during cheese making.

There has been a major thrust in the study of the microbial genetics of starter cultures both in industry and academia over the past 25 years (Johnson and Lucey, 2006). New starter strains of traditional starters that are genetically engineered to provide a variety of attributes are now available. These attributes include better phage resistance and specific growth profiles during cheese manufacture to improve the efficiency as well as the final consistency of cheese manufactured on a daily basis. Moreover, these starters provide specific fermentation profiles to produce customized cheeses with a variety of flavor and texture profiles, which has enhanced the use of cheese as an ingredient in different applications. For example, exopolysaccharide-producing bacteria enhance the texture of reduced- and low-fat cheeses (Hassan, 2008), salt-sensitive starters improve the flavor and texture of reduced- or low-sodium cheeses, and probiotic microorganisms can be incorporated into cheeses (Ong et al., 2006; Dias and Mix, 2008).

Coagulation

Milk coagulation is a key step in cheese production. Milk has a well-defined physical equilibrium between various constituents that mainly exists in three forms: emulsion, colloidal solution, and true solution. Milk lipids are present in an oil-in-water type of emulsion in the form of microscopic globules, varying from 0.1 to 22 μm in diameter. The colloidal phase contains casein micelles, calcium phosphate, and globular (serum/whey) proteins. Whey proteins are in a colloidal solution (diameter 4 to 6 nm) and the caseins are in colloidal suspension (diameter 50 to 300 nm). Lactose, acids, some inorganic minerals, vitamins and enzymes are present in true solution (diameter 0.5 nm).

The physical stability of milk is ascribed to the negative charge on casein micelles, which repel each other and do not aggregate nor form clumps. However, if the charge is neutralized by adding protons (H^+ ions) furnished by the addition of acid, casein micelles tend to stick to each other and milk begins to coagulate. This is acid-coagulation; it accelerates as the temperature of milk increases.

The second type of coagulation is induced by the highly specific proteolytic enzyme chymosin (Andren, 2003). Rennet is the

mode of coagulation in the vast majority of the world's cheeses. Normally, a casein micelle is composed of calcium-sensitive casein fractions (α_{s1}-, α_{s2}-, and β-casein) protected by an envelope of calcium-insensitive κ-casein. κ-casein is hydrolyzed as a result of the enzyme action. Accordingly, calcium-sensitive casein fractions are liberated and by interacting with calcium of milk, they aggregate and form coagulum.

As compared to other raw materials in cheese manufacture, the quantity of coagulating enzymes is rather small. For most cheese varieties, single-strength rennet has a usage level of 200 ml/1,000 kg of milk and the coagulation time is 20 to 30 minutes. The coagulating enzymes' preparations are obtained from animal, microbial, and vegetable sources, and they may take the form of a crude paste, powder, or liquid rennet. Most rennet preparations contain the coagulating enzyme chymosin or rennin with varying levels of pepsin.

Traditionally, rennet extracted from the abomasum (fourth stomach) of milk-suckling young calves was preferred. Because of scarcity of the calf rennet, or to accommodate religious beliefs, rennet-substitutes from other sources have been developed, including pepsins from swine, cows, and chickens. Microbial sources include *Mucor miehi, Mucor pusillus*, and *Endothia parasitica.* Clotting activity is ascribed to specific action on the phenyl alanine$_{105}$-methionine$_{106}$ linkage in the κ-casein molecule, whereas proteoltic activity refers to more random action.

Rennet substitutes generally display excessive proteolytic activity, resulting in reduced cheese yield and a bitter flavor in ripened cheeses. Synthetic chymosin is obtained by recombinant DNA technology. The genetic material from the calf abomassum is transferred to a host organism—*Escherichi coli, Kluyvermices lactis,* or *Aspergillus niger*—where it is used as a template to produce a coagulating enzyme identical to calf chymosin. Therefore, the recombinant chymosin is a product of fermentation technology, making it economically reliable and acceptable to most vegetarian, Jewish, Islamic, and Hindu consumers. It replicates the quality of cheese obtained by traditional calf rennet. Commercial rennet preparations typically are 50% calf chymosin and 50% bovine pepsin. Calf rennet preparations are 95% chymosin. Recombinant rennet preparations provide more consistency in heat tolerance and a dependable ratio of clotting activity and general proteolytic activity.

The clotting of milk by rennin at normal pH 6.6 is a three-phase reaction. In the primary stage, rennet cleaves a specific bond (phenyl alanine$_{105}$-methionine$_{106}$) in the κ-casein molecule, slicing it into para-κ-casein and soluble glycomacropeptide fractions. The hydrolyzed κ-casein can no longer hold the hydrophobic casein particles together. The Ca^{+2} ions commence coagulation of casein micelles in cheese milk when about 80% of the phenylalanine$_{105}$-methionine$_{106}$ bonds are cleaved. In the secondary stage, the micelles aggregate to form clusters, which lead to gel formation. Water, along with soluble constituents and fat, are trapped in the three-dimensional network. In the final stage the network continues to attain firmness. Cutting the gel is timed according to the type of cheese being produced. In soft, ripened cheeses, the gel is allowed to acquire more firmness, while for hard cheeses, the cutting process starts as soon as adequate firmness is achieved.

Residual rennet in cheese curd plays an important role in ripening. It is estimated that less than 15% of rennet used in cheese making is recovered in some varieties of cheese curd. Calf rennet is destroyed by the high cooking temperatures used in Swiss and Italian cheeses, but in cheddar cheese a significant amount of rennet survives and participates in proteolysis to yield desirable texture and flavor. Microbial rennet preparations are usually more heat resistant and tend

to survive high cooking conditions. Also, they end up in whey and may interfere with the functional properties of whey products; consequently, appropriate heat destruction procedures are necessary to ensure that the whey products are rennet free.

Handling and Use of Rennet in the Cheese Plant

It is necessary to understand the characteristics of rennet and its behavior under various conditions to achieve consistent coagulant activity, curd character, and cheese quality:

1. Heat-treatment of milk more severe than pasteurization causes heat denaturation and interaction of whey proteins with caseins. The heat-modified proteins do not form a strong gel. Therefore, high-heat treatment is detrimental for optimum coagulation and curd strength.
2. Homogenization of milk causes considerable changes in the reaction of milk to rennet as well as acid. The curd becomes whiter in color and more moisture is retained. Whey separation from the curd is reduced, and finer (softer) gel is produced due to the finer fat globule size. In soft cheeses such as cream cheese, homogenization is desirable because it enhances fat recovery in the cheese curd. However, in hard cheeses, it produces curd with a rubbery texture.
3. The optimum coagulation temperature for most cheeses is 30°C to 32°C (86 to 89.6°F). For Swiss varieties, it is 37°C (99°F). The curd is relatively fragile at temperatures below 30°C (86°F), thus making the curd-cutting step inefficient. Curd losses as fines are observed and the cheese yield is lowered. At temperatures below 20°C (68°F), coagulation is progressively weak but it is regained upon warming to 30°C (86°F). No discernible enzymatic or even acid coagulation of milk is observed at 4°C (39°F). Again, milk coagulation sets in on warming. Pasteurization of rennet destroys its activity.
4. The amount and structure of calcium in milk alter rennet activity. In general, Ca^{2+} ions accelerate clotting time and lead to the formation of firmer coagulum. It is customary to add 0.02% $CaCl_2$ to milk to assist in rapid coagulation. Too much calcium may cause corky body and other defects in cheese. Mastitis milk gives poor coagulation and should be avoided.
5. Dilution of milk or high-fat content leads to soft and fragile coagulum.
6. The pH of milk influences clotting time. Milk with lower pH (higher titratable acidity) clots faster. Rennet is more soluble at the lower pH developed during cooking of cheddar cheese curd (cheddaring process). Rennet retention is enhanced in curd under these conditions. Microbial rennet's solubility or retention is not influenced by low-draining pH.
7. Rennet is inactivated rapidly by alkaline pH values greater than 7. Because cheese color is normally alkaline, mixing rennet and color extract causes coagulation problems. Similarly, chlorinated water is inhibitory to rennet. As much as 40% of rennet activity is destroyed by three-minute exposure to 2 ppm of chlorine in water. Rennet should be diluted with 20 to 40 volumes of non-alkaline water immediately prior to addition to the vat. Agitating the vat for two to three minutes should disperse the enzyme thoroughly.
8. Agitation and vibration of milk during rennet setting/clotting leads to an undesirable weak curd.
9. Rennet should be stored in cold and dark place to preserve its activity.

Acid Coagulation

Acid coagulation, also known as isoelectric precipitation of casein, is used in ricotta,

Chhana, paneer and some Latin American cheeses (Chandan, 2003). In cottage cheese and cream cheese production, large aggregates of casein form clots *in situ* as a result of lactic acid production by the starter culture. The original pH of milk is around 6.6. As the acidification proceeds to pH 5.3, coagulation of casein commences. The coagulation is completed at pH 4.5 to 4.6, the isoelectric point of casein. The acid coagulation does not significantly depend on salt concentration of milk because it is triggered by the accumulation of free hydrogen ions in the system. At this stage, milk sets to a gel-like coagulum which can be processed further into curd and whey.

In comparison with enzymatic coagulation, acid coagulation results in considerably lower calcium and phosphorus in cheese curd. This observation is of important nutritional significance. On a dry matter basis, cottage cheese curd contains significantly lower calcium and phosphorus in comparison with enzymatic coagulated cheese curd, such as cheddar cheese. The reduced retention of these minerals in cottage cheese is attributed to progressive dissociation of calcium phosphate from the casein micelles as the pH of milk drops to pH 4.6, the isoelectric point of casein. Thus, a significant amount of milk calcium and phosphorus is leached out in cottage cheese whey (Clarke and Potter, 2007).

The recovery of major whey proteins (normally lost in whey) in cheese curd obtained by acid coagulation at ambient temperatures may be engineered by heating milk to elevated temperatures prior to acidification by direct addition of food-grade acid. In the manufacture of Indian cheese, Chhana and Paneer, heat treatment of milk at 90°C to 95°C (194°F to 203°F) for 5 minutes results in complex formation of κ-casein with β-lactoglobulin, along with heat-denaturation of whey proteins, making them insoluble (Chandan, 2007b, Aneja et al., 2002). They are then trapped in cheese curd along with casein clots. The incorporation of whey proteins in curd leads to higher cheese yield. Because whey proteins have a higher biological value than casein, whey protein retention in cheese curd improves the nutritional quality of cheese curd. Furthermore, the curd obtained by acidification at elevated temperatures retains more moisture, giving a harder texture and imparting a unique non-melting attribute. Such directly acidified cheeses are suitable for frying because they do not soften at frying temperatures and they retain their shape.

Cutting the Curd

After the coagulum is set in a horizontal (rectangular) cheese vat, a simple test is conducted to determine whether the coagulum strength is appropriate for cutting. A flat knife is inserted at a 45° angle to the surface of the coagulum and slowly pulled out. If the cut is clean and no curd sticks to the knife, the coagulum is ready for cutting. The setting temperature in Swiss cheese is higher than that in cheddar. Consequently, the Swiss cheese curd assumes a firm form quickly, necessitating cutting before it becomes too firm.

The size of cutting knife is chosen according to the particular variety of cheese. The curd size is related to retention of moisture in cheese. High-moisture cheeses such as soft, ripened varieties are cut with large 2-cm knives. Large curds are relatively fragile and produce more fines, leading to less retention of fat and nonfat milk solids in cheese. In some cases, the curd may be duly broken for dipping into forms/molds. Cheddar and washed-curd cheeses such as colby are cut by medium size (1-cm) knives. Small-curd size (resembling rice grains) leads to low moisture, as in Italian hard cheeses. Recovery of milk solids is higher in small-curd cheeses. High-setting temperature also assists in lower moisture retention.

Manual cutting of curd is done with a harp-shaped knife in which a series of stainless steel wires (resembling piano wires) are

stretched across a stainless frame in a vertical or horizontal fashion. The horizontal-wired stainless steel knife is pulled through the curd, followed by a vertical knife to complete the three-dimensional cut to form cubes of the curd. The cutting time is important to control the curd character and should be completed within five to 10 minutes. The cutting process is designed to increase the surface area of cheese cubes for enhancing syneresis or whey expulsion and efficiency of heat transfer during the cooking step.

After cutting, the curd is allowed to "heal" by leaving it undisturbed for 10 to 15 minutes. During this period, the curd cubes form new intramolecular linkages and they expel more whey and develop firmness. If a low-moisture, firmer cheese is needed, the curd is agitated for 30 minutes before the cooking phase. This step helps to avoid surface toughness of the curd cube. The cutting quality can be determined by how much fat is lost in whey (normally 0.2% to 0.3% fat).

Cooking the Curd

Cooking refers to application of controlled heat to the curd cubes. Hot water is circulated in the cheese vat jacket to transfer heat to the curd-whey mixture while the curd is stirred at a slow speed. The final temperature is 37°C to 41°C (98.6°F to 106°F) for many cheeses; it is as high as 53°C (127°F) for Swiss and parmesan cheese. The final temperature is consistent with the thermal sensitivity of the starter cultures used in a cheese variety. The rate of heating the curd also depends on the cheese variety. In general, heating the whey-curd mixture starts slowly; stirring is likewise slow to preserve the integrity of the fragile curd at this stage. As time passes, and the curd assumes a firmer texture, the rate of heating picks up until the desired cooking temperature is achieved in 30 to 40 minutes. It is customary to cook some fresh cheeses such as cottage, cream, and Neufchatel to 52°C to 60°C (126°F to 140°F) to facilitate syneresis and extend product stability.

After the cooking temperature is attained, the cheese vat agitation is set to high speed and the curd-whey mixture is stirred vigorously for another 45 to 60 minutes to promote more syneresis of whey. During the cooking and afterward, the acid production by the starter culture continues and the titratable acidity of whey increases (pH decreases), further promoting syneresis of the curd. The status of colloidal calcium phosphate associated with casein begins to change by becoming soluble as acidity builds up in whey. In fresh cheeses, the pH drops to 4.6 to 4.7 and all of the colloidal calcium ends up in whey. However, in renneted curd varieties, the pH of curd is higher (greater than 5.3) and some colloidal calcium is retained in the cheese curd. Depending on the pH developed after cooking, the cheese curd retains varying levels of calcium, which affects the cheese texture accordingly. In general, fast acid development results in low-pH curd, thereby causing low calcium retention in cheese curd and leading to a crumbly texture, as in Cheshire cheese. On the other hand, as in Swiss cheese, slow acid development (higher pH) results in a higher calcium content in the curd and a more elastic and rubbery texture.

Lactose of milk is partitioned between whey and the cheese curd. Its content in curd is proportional to moisture content of the curd. Equilibrium is established between curd lactose and whey lactose. Because lactose is metabolized to lactic acid, its content in curd provides potential fermentation ability and acid development. After the curd and whey are separated, the lactose in curd is metabolized quickly. However, if the curd and whey stay together longer, more acid is generated, causing changes in the physical properties of the curd. The buffering capacity of the curd is reduced due to acidity-induced loss of calcium and phosphate to whey. Under such conditions, the lactose in curd stays intact longer, which in turn fer-

ments rapidly without the buffering capacity of calcium and phosphorus. Thus, the curd develops an acidic flavor and the body becomes weak and pasty.

Draining the Whey and Milling the Curd

When the cooking step has been completed and the desirable acidity has been recorded (pH of whey 6.1 to 6.4), the whey is physically removed from the vat. The curd is allowed to settle to the bottom of the vat and a screening device is fitted in the discharge end of the vat. On opening the valve, clear whey exits from the vat, leaving a heap of curd behind. The draining time from all vats (typically 20 minutes) should be fairly uniform to maintain quality of the cheese. The curd is trenched on the sides of the vat to facilitate further draining. The curd is allowed to stick together (matting) to form loaves, while acidity builds and whey acquires near-clear character. In cheddar, American, and pasta filata cheese varieties, the final pH should be 5.2 to 5.4. When the proper acidity level is attained, the cheddar cheese loaves are ready for milling into small cuts and salting. The pasta filata cheese loaves are fed into hot water stretching and molding equipment, where the curd is softened, melted, and stretched in hot water and then exits in a particular shape. These forms are immersed in cold brine for cooling and salting.

Whey separation in automated cheese vats is carried out by pumping curd along with whey onto a draining and matting conveyer belt, where it is allowed to reach proper acidity. The fused curd mat is then mechanically cut, salted, and conveyed to the hooping station for shaping into cheddar blocks and barrels. Salting occurs after hooping and pressing in several other cheese varieties. Whey is processed further to manufacture important ingredients such as dry whey, lactose, and whey protein concentrates (Kilara, 2008).

Hooping and Pressing

In soft and semi-soft cheeses, the curd and whey are dipped into perforated molds and hoops of selected shapes and sizes. As whey drains, the curd settles. The hoops should be turned upside down at regular intervals to ensure better draining of whey and formation of a smooth plastic mass of uniform shape. The molds and hoops are selected to form discs of various sizes of small wheels or slabs of cheese. The cheese forms are removed from the molds and hoops and immersed in brine for cooling and salting.

In Swiss and Dutch type cheeses, prior to draining, the curd is pressed under whey to eliminate trapped air and liquid, to obtain a smooth texture. For certain cheese varieties (Brick, Muenster), the curd is washed with water to reduce the lactose content. After draining up to 67% of the whey, fresh water is added to the vat to replace the drained whey and the curd-water mixture is agitated for about 15 minutes. The washing step results in higher moisture retention in cheese while achieving a pH of 5.0 to 5.2. The water temperature influences the moisture retention in cheese curd. Gouda cheese curd is washed with hot water to dry out the curd.

The curd is filled into hoops; in hard cheese varieties it is subjected to hydraulic pressure to fuse the curd into a single block. For cheddar, traditionally 170 kPa of pressure is applied for several hours. Warmer curd requires lower pressure. Little or no pressure is needed for soft cheeses. A small amount of whey may be expelled at this stage. Vacuum treatment can be applied before or after pressing to reduce/eliminate mechanical openings in the blocks.

Salting the Curd

Salt (sodium chloride) incorporation in cheese curd is another key step in cheese production. Sutherland (2003) has described

the role of salt in cheese manufacture. Salting may be accomplished by adding crystalline salt to the curd prior to pressing, as in cheddar, colby, and Monterrey Jack cheese varieties. Another technique of salt incorporation involves immersion of pressed cheese blocks, wheels, or discs in a cold brine solution containing approximately 23% sodium chloride. Coarse salt is rubbed on the surface of certain cheese varieties. In each case, the contact time of the salt with the cheese curd is designed to permit adequate diffusion of the salt into the interior of the cheese bulk. Cheeses that are salted in brine include Gouda, Edam, Swiss, camembert, Brie, mozzarella, parmesan, Romano, provolone, and blue-veined varieties. In rare cases, both dry salting and immersion in brine may be practiced. In mechanized cheese making, brine solution is injected into the cheese curd.

Most cheese varieties contain 1.2% to 2% salt. For cheddar cheese containing 38% moisture and 1.2% salt, the effective salt level would amount to 3.2% salt in the aqueous phase. Similarly, in mozzarella containing 50% moisture and 1.2% salt, the effective level of salt would be 2.4%. For pickled cheese, such as feta, much higher levels of salt are used. The concentration of salt in cheese curd and the manner of its incorporation play an important role in the characteristic cheese flavor and texture development.

The function of salting is three-fold. First, it imparts to cheese an agreeable taste. Second, it directs the fermentation pattern of the cheese ripening process by selectively inhibiting undesirable microorganisms and controlling the growth rate of ripening organisms. Third, the salting process results in syneresis and removal of additional whey from the cheese curd to impart typical textural quality to cheddar cheese. The regulation of moisture in cheese subsequently affects the fermentation process, leading to specific flavor production in cheese.

The quality of salt used in cheese plant must conform to the specifications defined in an industrial standard publication similar to Food Chemicals Codex. It should be free of additives, pathogenic microorganisms (staphylococci, salmonella, coliforms), and environmental pollutants. The suggested particle size should range from 20 to 70 mesh. The typical total bacterial count should not exceed 1,000 CFU/g, and the yeast and mold count should not exceed 100 CFU/g. Extraneous matter measured by the USDA sediment test should not exceed 1.5 mg (No. 2 pad), using a 250-g sample of salt. To avoid moisture absorption and caking, the salt should be stored in sealed, plastic-lined bags under rodent-proof conditions.

Factors to be considered for ensuring precision and uniformity of salt incorporation are discussed below in relation to the salting method.

Dry Salting

Dry salting milled curd establishes quick distribution of the salt, whereas rubbing of salt on cheese surface appreciably delays the equilibrium conditions. The amount of salt added to cheese curd is determined by the weight of curd, the desired salt level in the cheese, and the size of the curd particles. The salt penetration is accelerated by lower pH and higher moisture content of curd, but is slowed by larger curd size and delays salting of milled curd.

For a given variety of cheese, the yield of curd may be considered relatively constant. Accordingly, the amount of salt added to the curd varies with the quality of the curd after milling. In general, 2.5+/- 0.5 lbs of salt/1,000 lbs of milk is used in cheddar and American cheese making. Approximately 60% of the added salt is retained in the cheese. Assuming 95 pounds of cheese curd obtained from 1,000 pounds of milk, this level corresponds to 1.3% to 1.9% in cheese.

The salt dissolves in the aqueous phase of cheese. Therefore, in cheese containing 38% moisture, the effective salt concentration in solution is 2.63 × the salt level of cheese, which calculates to 4.2%.

The effective salt concentration controls the pattern of cheese fermentation during ripening. Lactic acid production is also slowed considerably, which in the long run helps to balance moisture retention in cheese. Concomitant with the penetration of salt in cheese, clear whey is exuded from milled curd. This is related to the osmotic effects of salting. Coarse salt is preferred because it diffuses slowly. Finer grains dissolves too quickly and the salt is easily lost in the whey. To facilitate slow absorption, coarse salt is applied in three installments.

Evidently, sodium ions at the normal salt level in cheese are toxic to many undesirable organisms, especially in the early stages of salting when the salt level is extremely high on the outer surface of the cheese particles. It is common practice to use a higher salt level (3 lbs/1,000 lbs of milk) in cheese curd that has excessive moisture or acidity. Similar action is warranted if the curd develops gassiness or off flavors.

It should be remembered that over-salting leads to harder and drier cheese with restricted ripening effects. On the other hand, lack of adequate salt causes higher moisture retention in cheese and accelerated breakdown of protein and fat with severe body and flavor defects. This results from changes in the ecological balance of the microflora during cheese ripening. Salt in optimum levels also solubilizes constituents of cheese as well as microbial cells, thereby enhancing enzymatic reactions during curing. The presence of salt in cheese may be involved in controlling toxin production by *Clostridium botulinum.* However, other food poisoning organisms such as *Staphylococcus aureus* are tolerant to a 5% to 10% salt level.

Brine Salting

Brine salting involves dipping cheese blocks in a sodium chloride solution containing 18% to 26% salt and 0.1% $CaCl_2$. The pH of brine is adjusted to 5.2 to 5.6 to protect the surface appearance of the cheese form. Cheese blocks and other forms are immersed in brine at 9°C to 16°C (48°F to 60°F) for penetration and equilibration of salt. Dry salt is sprinkled on the surface of the floating cheese blocks. The period of immersion depends on the temperature of the brine, the recirculation rate of the brine, and the surface area/weight unit of cheese. The optimum salt concentration of cheese is 1.75% to 2.25%.

The time period for brine treatment varies with the size of the cheese block. Small cuts and sizes (250 to 350 g) are dipped for one to four hours for proper salt penetration. For larger sizes, for example, 3 to 5 kg, it may take two to four days, and for even larger sizes (20 kg), the time must be extended to at least five days.

Brine tanks are constructed of materials resistant to salt corrosion. Stainless steel tanks are likely to be corroded by salt in a relatively short time. Accordingly, fiberglass or suitable plastic tanks are common. Freshly made brine should be checked for its strength using a floating hydrometer, which measures the specific gravity of brine. The relationship between the percentage of salt and the specific gravity of the brine determines the salt strength of the brine. A salometer (salimeter, salinometer) is another floating instrument for checking the salt strength of brine. It is calibrated in degrees in which 0° represents the specific gravity of pure water. A salometer reading of 100 is equivalent to saturated brine at 3.3°C (38°F), which corresponds to 26.4% salt concentration or specific gravity of 1.204.

The brine should be checked daily to ensure proper strength, and fresh salt should be incorporated to make up for any dilution.

The brine accumulates cheese solids during successive use, which may encourage microbial growth. If the standard plate count on the brine exceeds 100,000 CFU/g, it is advisable to pasteurize or boil the brine solution. Some manufacturers add 100 to 500 ppm of chlorine weekly to control microbial contamination. Recirculation of brine facilitates uniformity of salt strength. A cheesecloth filter in the line helps to trap and discard accumulated cheese solids.

The hydrometer/salometer readings on used brines should be corrected for the contribution of cheese solids to the readings. The actual salt strength may be 1% to 2% lower than the strength calculated from the readings. Analytical measurements of salt using silver nitrate titration should be periodically undertaken to verify the discrepancy between the actual sodium chloride concentration and salt strength observed with a hydrometer.

The brine-salted cheese blocks are allowed to dry out by 24-hour storage in a refrigerated room prior to packaging and ripening.

Preparing Cheese for Ripening

Mold-surface-ripened cheeses are sprayed with suspensions of white mold *Penicillium camemberti/candidum*. Blue-veined cheese blocks are drilled with vertical holes and then sprayed with blue mold, *Penicillium roqueforti*, to encourage the growth of the mold in the interior of cheese.

Treatment of cheese blocks prior to ripening includes formation of the rind, bandaging, waxing, and film wrapping. Soft cheeses form a rind during ripening by the growth of bacteria and molds as well the loss of moisture from the surface. The rind hardens as ripening proceeds, and gives cheese a rigid surface for ease of handling. Various rind treatments are used to prevent cracking, mold growth, and dehydration of rind. These include wrapping in leaves, ash, or spices, repeated washing/salt treatments, or vegetable oil/butter oil treatments. The rind may be coated with wax or resinous material to prevent mold and mite infestation. Some cheeses are preserved by smoking. Feta and some other cheeses are stored in drums containing brine. In Muenster cheese, washed rind is smeared with *Brevibacterium linens.* Some time mold inhibitors (sorbate, propionate, pimaricin) are used in the wax, emulsion or film envelope formed around the cheese.

In earlier times, cheese underwent the process of bandaging and dressing. Bandaging involves the application of a calico or cheesecloth bandage followed by treatment of the cheese with greased muslin. The structure was either sewn or glued on with a flour paste. Dressing the cheese involved the application of a wax, oil, or fat to develop a protective rind. Cheese with rind reduces the yield of saleable cheese and many operations have switched over to rindless ripening.

A large majority of cheese is now packaged in films and foils. Tight wrapping with certain films has been used for large ripening blocks. Shrink films also are used. Some cheeses are dipped, sprayed, or coated with molten colored waxes and resins. The waxes coat the surface of cheese itself or may be combined with tight wrapping with bandages of cloth or plastic film. Cheese may be wrapped first, followed by dipping in molten paraffin wax. Alternatively, cheese may be waxed prior to wrapping. Waxed cheeses should be ripened under high humidity conditions because the wax coating is inherently more prone to moisture loss than film wrapping. Wax and film wrapping materials may be impregnated with anti-mold agents such as sorbate, propionate, or pimaricin to prevent growth of mold in cheese blocks. Larger cubes or drums of cheddar (500 to 640 lbs) are ripened in barrier films that are impervious to gas migration and moisture loss. Special films are used for ripening rindless Swiss cheese because of considerable evolution of CO_2 during ripening. Such films are designed to absorb the gas, whereas others have low

barrier properties to permit escape of the gas. The moisture barrier packages are vacuum-treated to expel air and are often flushed with CO_2 or N_2 gas to prevent mold growth during ripening. Flushing with CO_2 offers the advantage of solubility characteristics of the gas, making the package tight enough to cling to the surface of the cheese. Shrink films also give skin-tight packaged after dipping them in hot water or by passing them through a steam chamber. Packages containing cheese curd or cheese shreds are flushed with nitrogen to avoid fusion of curd particles.

Ripening

Pressed cheese blocks are protected from moisture loss and growth of undesirable bacteria and molds by wax coating, rind formation, enrobing in special emulsions, or vacuum wrapping in plastic films. Rind formation is characteristic of Italian cheese but is also practiced in some cheddar cheese production. Cheddar and American cheeses are ripened in film-wrapped blocks. The packages are then allowed to ripen by placing them in ripening rooms with controlled temperature. For some cheeses the humidity also is controlled. The ripening period varies from zero to two to five years for hard varieties. During ripening, the major constituents of cheese (lactose, fat, protein, and metabolic products of culture) growth are further broken down to form the typical cheese flavor and texture.

Cheese ripening is associated with the action of several enzymes and cultures. Plasmin and lipoprotein lipase are the milk enzymes involved. Plasmin survives pasteurization of milk and cleaves caseins, especially in Swiss cheese. However, the role of plasmin is minimal in cheese made from ultrafiltered milk that contains β-lactglobulin, an inhibitor of plasmin. Lipoprotein lipase hydrolyzes milk fat to generate flavorful compounds, but its role is restricted only to cheese made from raw milk.

Residual calf rennet and recombinant chymosin hydrolyze casein fractions and produce precursors of cheese flavor. In this regard, chymosin specifically attacks α_{s1}-casein, whereas β-casein is not affected by rennet. Starter cultures proliferate in the early stages of ripening, reaching as high as 500 CFU/g in three to four days. However, their numbers dwindle soon after, reaching 30 million/g after four weeks. The dead cells release enzymes, which continue hydrolyzing caseins and milk fat. The leuconostocs generate CO_2 from inherent lactose and citrate to create small eyes and produce diacetyl and other potent flavor compounds. In Swiss cheese varieties, propionibacreia are allowed to grow by keeping cheese blocks at 20°C (68°F) for about three weeks, resulting in enough CO_2 production for generation of large eyes in the cheese body.

High humidity (90% to 95%) and air circulation (for oxygen supply) are required in surface-ripened cheeses such as Camembert and Brie to encourage the growth of molds *Penicillium camemberti and Penicillium roqueforti*. Similarly, in smear-ripened cheeses, *Brevibacterium linens* is encouraged to grow by maintaining high humidity. Simultaneously, the growth of mold is restricted by repeated surface washing. In addition to starter cultures, non-starter lactic acid bacteria (especially lactobacilli and pediococci) also play an important role in cheese ripening.

Several enzyme preparations are available for accelerated ripening. They are derived from dairy or non-dairy sources, most of them lipases and proteases. Enzyme capsules also are designed to liberate enzymes at certain stage of ripening. Heat or freeze-shocked proteolytic cultures have been used as well.

The chemistry of cheese flavor is reviewed by several authors (Fox, 2000, 2003b; Singh and Cadwallader, 2008). The origin of the cheese flavor profile is summarized below.

Lactose

Lactose is first broken down via the glycolytic pathway by starter bacteria to L-lactic acid. In cheddar cheese types, most lactic acid is produced in the cheese vat, whereas in other cheeses, acid formation occurs in the curd blocks. Nearly all of the lactose is metabolized within a day of cheese making in the Swiss variety because of efficient draining of whey. Furthermore, L-lactic acid gives rise to propionic acid, acetic acid, and CO_2 by propionic acid bacteria. The CO_2 gas pressure creates "eyes" in the cheese texture. In the surface-mold ripened cheeses (Camembert, Brie), lactose breaks down on the surface to H_2O and CO_2, allowing the mold growth with the resulting pH increase in cheese. In washed curd varieties, especially Gouda, salting is delayed, which leads to quick utilization of lactose. Salting is done early in colby, which delays the total utilization of lactose. During ripening, non-starter lactic acid bacteria convert L-lactic acid to DL-lactic acid, which further degrades to acetic acid by lactobacilli and pediococci. Cheese ripened for an extended period may display a white surface due to the formation of insoluble calcium lactate crystals.

Citrate

Citrate is naturally present in milk. During cheese making, 0.2% to 0.5% citrate is retained in the cheese curd (Singh and Cadawallader, 2008). The Leuconostocs and *Lactococcus lactis* biovar. *diacetylactis* produce flavor compounds from citrate, acetate, diacetyl (2,3 butanedione), acetoin (3-hydroxy-2-butanone) and 2,3 butandiol. Carbon dioxide is also produced, which generates small eyes in Dutch-type cheeses.

Milk Fat

Milk fat is the source of a host of flavor compounds. It is hydrolyzed by lipases to yield fatty acids which form substrates and undergo biochemical transformation to yield an array of flavor compounds. For example, butyric acid, long-chain free fatty acids, several lactones, and others form the backbone of the flavor profiles of Camembert, Brie, Roquefort, blue, Stilton, feta, Romano, and provolone cheeses. The aroma of mold-ripened cheeses is attributed to degradation products of fatty acids, methyl ketones, and secondary alcohols. Milk-fat-derived γ- and Δ-lactones participate in the overall flavor profile of cheddar cheese.

Caseins

Caseins are the substrates for production of an array of flavor compounds. In the early stages of ripening, hydrolysis of caseins is carried out successively by rennet, plasmin of milk, culture proteinases, and peptidases to produce peptides and amino acids. The peptidases release large varieties of amino acids which degrade further to amines, α-keto-acids, hydroxy acids, sulfur compounds, aldehydes, alcohols, esters, and thio-esters. The level and ratios of many known and unknown compounds orchestrate typical flavor profiles. The aroma is partially ascribed to dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide, which arise from methionine. Although a better understanding of flavor profiles exists for several cheese varieties, it is still not possible to duplicate cheese flavor by mixing various chemicals in a flavor cocktail.

Proteolysis is also responsible for textural modifications during ripening. As ripening proceeds, protein degradation intensifies, and water-soluble nitrogenous compounds register a significant increase. Their level is generally used as an index of cheese maturation.

Control of Ripening Conditions

Moisture

Higher moisture early in ripening of cheddar cheese is likely to accelerate ripening and

impart off flavors to the cheese. The water activity generally increases with ripening.

The percent moisture in nonfat solids of cheese (MNFS) is considered a better parameter to explain ripening patterns. It is calculated as follows:

$$\text{MNFS} = \frac{(\%\ \text{cheese moisture})}{(100 - \%\ \text{cheese fat})} \times 100$$

In New Zealand, high-quality cheddar cheese is targeted to have a MNFS percentage in the range of 50% to 57%. At 10°C (50°F), the MNFS is observed to decrease from 56% after three to four months of ripening to 53% at six to seven months of ripening. Critical parameters for control of MNFS include pH (at the whey draining and salting stages) and cooking temperature and fat in dry matter.

Salt Concentration

A better parameter for quality control is salt in moisture (S/M) content of cheese. It is calculated as:

$$\%\ \text{S/M} = \frac{(\%\ \text{salt in cheese})}{(\%\ \text{moisture in cheese})} \times 100$$

High S/M controls the acidity development by starter bacteria; low pH discourages the growth of undesirable bacteria and influences proteolysis and generation of flavor compounds. The target level of the S/M percentage is 2.5% to 6% for cheddar cheese.

Fat Content

Fat content makes cheese soft. When the fat content is high, whey drainage is impaired. Fat in dry matter (FDM) is considered a better quality control parameter. It is calculated as follows:

$$\%\ \text{FDM} = \frac{(\%\ \text{fat in cheese})}{(\%\ \text{total solids in cheese})} \times 100$$

For cheddar cheese, the target value is 50% to 56% FDM. This value also is important to meet regulatory requirements. As the FDM increases, the MNSF tends to increase. The FDM of cheese is controlled by the casein:fat ratio in milk.

Acidity/pH

Acidity/pH control is critical at the time of cutting, draining, and milling, as well as after one day and seven days of ripening (Hill, 2009). During the first week of ripening, the pH of most cheese varieties should be 5.0 to 5.1. Normally, the pH should increase at the end of the ripening period. The surface of mold-ripened cheeses registers an increase from pH 4.6 to pH 7.0. This increase is ascribed to formation of alkaline fragments of casein. The increase in pH enhances the activity of proteases and lipases.

Ripening Temperature

For a given cheese, the temperature of ripening is also a factor of cheese quality. For cheddar, it is recommended to ripen the blocks at 4°C to 10°C (39°F to 50°F) for the first few weeks, followed by ripening at 8°C to 10°C (46°F to 50°F). A low temperature slows down the starter as well as non-starter organisms to control pH within the desirable range (above 5.0) and to reduce formation of off flavors. Other varieties are ripened at 10°C to 15°C (50°F to 59°F), whereas surface-ripened cheeses are ripened at 11°C to 15°C (52°F to 59°F).

Humidity Control

Some cheeses, especially the surface-ripened varieties, require high humidity and air circulation to encourage the growth of molds and yeasts. The humidity requirement for surface-bacterial-ripened cheeses is 90% to 95%, whereas fungal-ripened cheeses need 85% to 90%. Cheese with a dry rind needs 80% to 85% humidity.

Factors for Optimizing Cheese Yield

Cheese yield is defined as pounds of cheese obtained from 100 pounds of milk. It depends on the milk and cheese composition, as well as the added salt level. Generally, to achieve high yields of cheese, milk with high levels of casein and fat are selected. Milk should be of excellent microbiological quality. Milk with high bacteria counts usually displays enzymatic breakdown of protein, thereby reducing casein content leading to poor cheese yield. In addition to milk quality, processing parameters affect the yield of cheese. Attention should be focused on the coagulum cutting procedure, heating rate during cooking, timing of salting after milling, temperature during pressing, washing of curd, and the heating and stretching step in mozzarella cheese.

The cheese-making process partitions major constituents of cow's milk into cheese and whey. For example, in cheddar cheese, approximately 4% of the water, 93% fat, 75% protein (96% casein, 7% whey protein), 35% minerals, and 3% of the lactose of the milk are retained. On the other hand, approximately, 96% of the water, 7% milk fat, 25% protein (4% casein, 93% whey protein), 65% minerals, and 97% of the lactose of the milk end up in whey.

Figure 10.5 illustrates a mass balance diagram for cheddar cheese. For cost control, it is useful to monitor incoming milk, cheese, and whey fractions for fat, protein, mineral, and moisture percentages. This can assist in auditing the plant's performance with respect to typical retention values. It is advisable to develop the data relevant to a specific cheese operation in relation to cheese varieties during the course of a year. Undue loss of fat and protein in whey should be investigated as a managerial tool. The quality of cheese must be maintained while steps are taken to optimize the yield of cheese.

Controlling moisture and fat in cheese is necessary from legal, cost, and quality standpoints. It also is important for cheese output in a cheese plant.

Moisture Control

The U.S. Food and Drug Administration (FDA) regulations require a minimum of 39% moisture in cheddar cheese. Theoretically, an increase of 2% moisture in cheddar cheese from 36% to 38% can lead to significantly more output, as shown below.

Assuming 75% retention of milk protein, 93% retention of fat, 30% retention of lactose, and 35% retention of minerals in cheese, cheese will have dry matter as follows:

$$\text{Protein} = 0.75 \times 3.2 = 2.40 \text{ lbs} \ldots 1$$

$$\text{Fat} = 0.93 \times 3.6 = 3.35 \text{ lbs} \ldots 2$$

$$\text{Lactose} = 0.30 \times 4.7 = 0.14 \text{ lbs} \ldots 3$$

$$\text{Minerals} = 0.35 \times 0.8 = 0.28 \text{ lbs} \ldots 4$$

Adding lines 1 through 4, the dry matter yield is 6.17 lbs.

Assuming 36% moisture in cheese and 1.55% salt absorbed by cheese curd:

$$\begin{aligned}&\text{The cheese yield}\\ &= 100 \times (\text{dry matter weight}) / \\ &\quad (100 - \%\ \text{moisture} - \%\ \text{salt})\\ &= 100 \times 6.17 / (100 - 36 - 1.5)\\ &= 100 \times 6.17 / 62.5 = 9.87\%.\end{aligned}$$

In another scenario, assume the moisture level in cheese is 38%. Then:

$$\begin{aligned}&\text{The cheese yield}\\ &= 100 \times (\text{dry matter in cheese}) / \\ &\quad (100 - \%\ \text{moisture} - \%\ \text{salt})\\ &= 100 \times 6.17 / (100 - 38 - 1.5)\\ &= 100 \times 6.17 / 60.5 = 10.20\%\end{aligned}$$

In this illustration, in a cheese operation processing 500,000 lbs of milk per day, there is an opportunity to generate an extra 1,650 lbs of cheese on a daily basis by tightening the moisture in cheese from 36% to 38%.

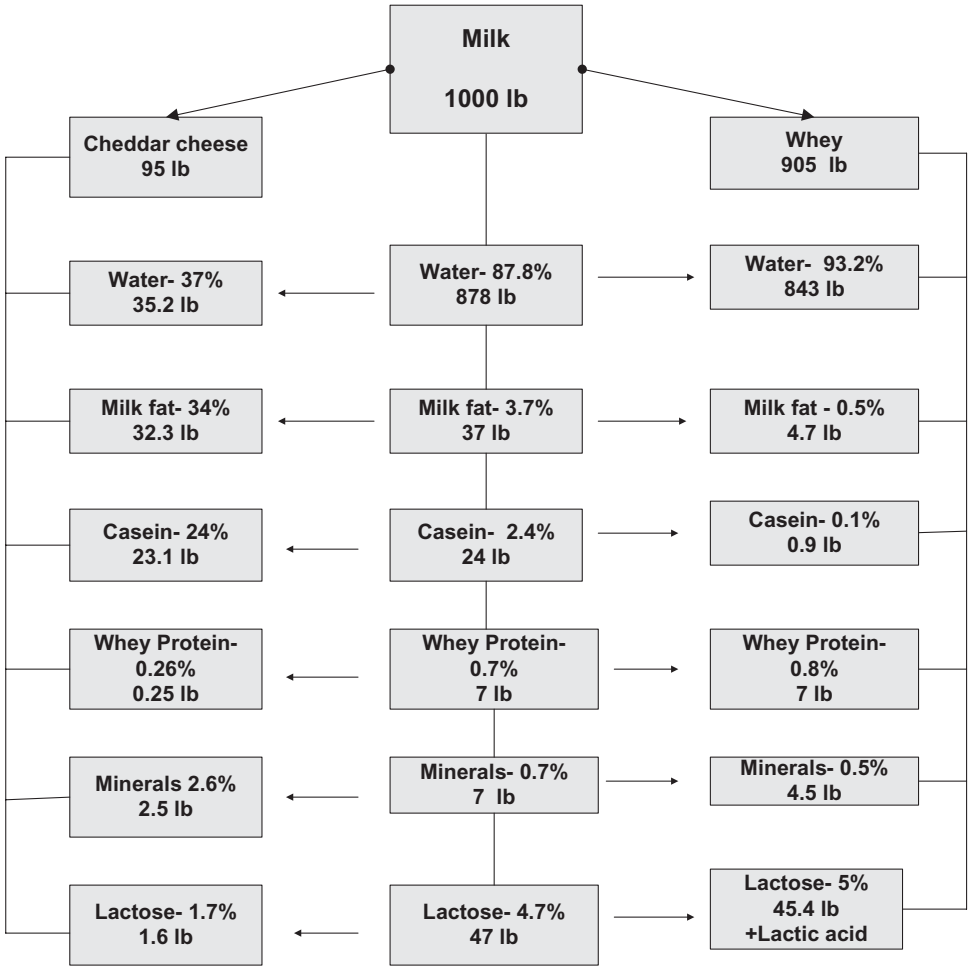

Figure 10.5. An example of a mass balance diagram for the cheddar-cheese-making process, showing partitioning of major constituents of milk into cheese and whey.

The moisture content of a cheese variety plays a role in imparting a characteristic flavor and texture. Water functions as a medium for microbiological growth and the accompanying biochemical transformations of the major curd constituents during cheese ripening. Higher moisture is favored by cutting coagulum into larger cubes; curtailing the cooking time, temperature, and acidity development, and reducing the salt level. In addition, the cheddaring procedure may be modified and pressure may be reduced in the cheese press. Steps may be taken to control moisture loss during extended periods of ripening.

Because the fat standard in most cheeses is defined in terms of dry matter or total solids content, the moisture content has an important bearing on the fat standard.

Fat Standard

Depending on the cheese variety, the milk composition—especially the casein : fat ratio—is a key factor in meeting fat and moisture standards. Although cheese fat and moisture

content are legal obligations, they are important criteria of functionality (performance) and application in food processing. Consequently, processing parameters should be examined with a goal of minimizing fat losses in whey.

The cheese yield for cheddar cheese can be predicted by the classical Van Slyke and Price formula:

$$\% \text{ Yield} = [1.09 \times (0.93\text{F} + \text{C} - 0.1)]/(1 - \text{M})$$

Where F = % fat in milk,

C = casein content of milk,

M = moisture content of cheese.

It is assumed that casein losses in whey are 0.1%, and 1.09 is a factor representing recovery of minerals, lactose, and other whey components in cheese.

Assume that milk has 3.7% fat and 2.4% casein, and cheese has 37% moisture or 0.37 moisture fraction:

$$\text{Predicted yield} = [1.09 \times (0.93 \times 3.7 + 2.4 - 0.1)]/(1 - 0.37)$$
$$= [1.09 \times (3.441 + 2.3)]/0.63 = 9.933\%$$

This formula has drawbacks but it is advisable to calculate one's own predicted yield from daily analytical data.

Milk composition data should include the percentages of fat, protein, lactose, ash, and total solids in incoming milk. Similar data should be collected on cheese and whey.

The salt content of cheese also should be analyzed. The composite data helps to monitor the percentage of loss of fat and solids in whey and the percentage of recovery of fat and solids in cheese. Data collected over a year should reveal seasonal variation in milk and the actual percentage of yield as well as composition and salt content of the cheese variety. It also helps in correlating peak cheese yield with percentage of transfer of fat, protein, and salt in a given cheese variety, as well as the processing parameters to achieve the best yield.

Mechanization in the Cheese Industry

As indicated earlier in the chapter, there have been immense advances in the automation and mechanization of cheese manufacture (Robinson and Wilbey, 1998; Bylund, 2003). Figure 10.6 indicates a schematic flow chart of present day mechanized cheddar cheese manufacture.

The use of automated cheese milk standardization equipment and advances related to cheese cultures has been highlighted in previous sections. This section describes some of the modern technologies presently practiced in the industry for cheese manufacture.

Coagulation, Cutting, and Cooking the Curd

Cheese making has traditionally involved square or oval double-jacketed open vats to perform the coagulation of cheese milk, followed by subsequent cutting and cooking of the curd. Open vats are still used in smaller cheese plants as well as for artisan cheese manufacture. However, commercial manufacture of cheese is now done in enclosed double jacketed vats with mechanical cutting and stirring devices. These vats have cutting and stirring blades that rotate on a shaft, either vertically or horizontally. The rotation is controlled by a variable speed drive, ranging from 2 to 15 rpm as desired.

Various manufacturers have developed various patented vat designs. Some of the present ones include Double O Vat® (cutting blades on a vertical shaft) and HCV® (horizontal cheese vat) from Tetra Pak (Vernon Hills, IL) and Horizontal II Vat® (cutting blades on a single horizontal shaft) (Stoelting, Kiel, WI). These vats can hold up to 70,000 lbs of milk. Horizontal vats are gaining popularity because their use leads to an improvement in cheese yield (less fines production and less fat losses) due to improved cutting and stirring designs. They also have been found to

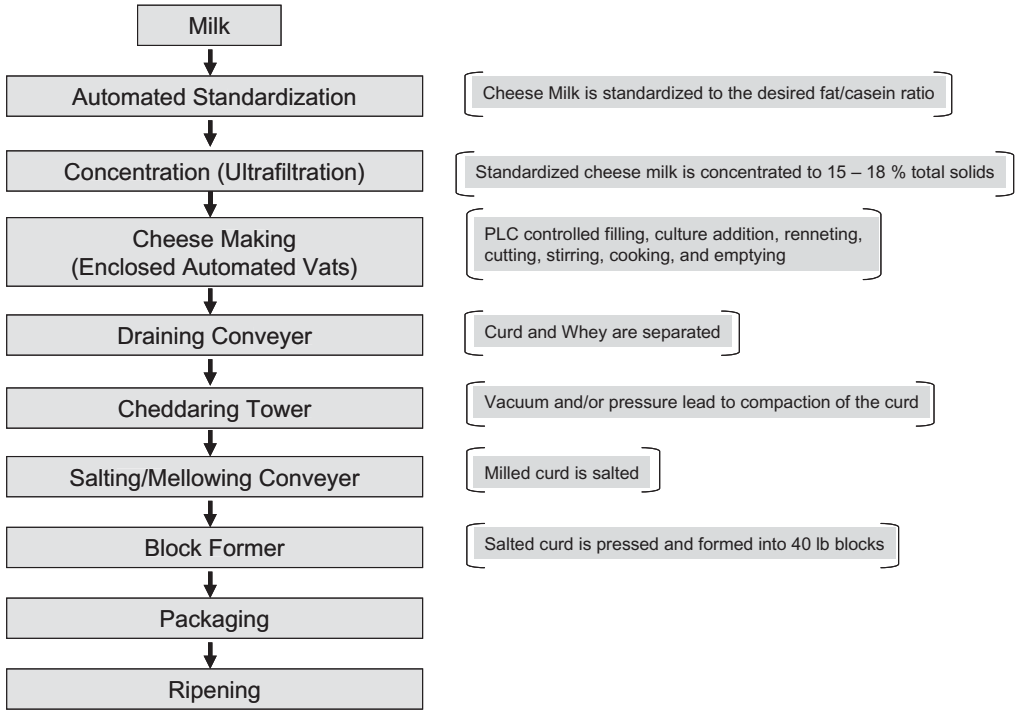

Figure 10.6. A schematic flow chart of present day cheddar cheese manufacture.

be better suited to handle cheese milk with higher total solids content (Johnson and Lucey, 2006).

These vats are equipped with computer controls to automatically follow the sequence for filling, culture addition, renneting, cutting, stirring, cooking, and emptying. Almost all of the enclosed vats are equipped with an inline pH probe to monitor the milk and curd/whey pH during the various stages of cheese manufacture and most of them include an automated gel-strength analyzer (Stoelting, Kiel, WI) to determine the end point of cutting.

Challenges associated with automated cheese vats that may lead to production down time as well as inconsistencies in the final product include ineffective cleaning-in-place (CIP) systems and the need for regular maintenance of pH probes and gel-strength analyzers to ensure their effectiveness on a daily basis.

Curd-Whey Separation

The whey must be separated from the curd after cutting of the curd and cooking. This is achieved by various mechanized whey separation devices, as discussed earlier. Whey separation devices typically include a drain table and/or vibrating screens to remove the whey from the curd. These devices are sometimes followed by centrifugal separators to remove and recycle cheese fines before the whey is transferred for processing. In the case of cheeses such as Swiss, Emmental, and havarti, the drained curds are salted and transferred to hoops and pressed. However, in the case of cheddar (which must undergo the cheddaring step to acquire its

unique body), various specialized pieces of equipment have been designed and are now used in the industry.

Cheddaring, Draining, and Salting

The two basic principles of cheddaring involve stretching and matting of the curd. Once again, mechanized equipment has been designed to complete these steps. One method is endless flexible conveyer belts in which curd (after whey drainage) travels on moving conveyers and is consequently stretched and matted by the conveyers running at different speeds.

The most common method of cheddaring uses Cheddaring Towers (Cheddar Master®, APV, Getzville, NY). Cheddar Towers employ a vertical column approximately 11 m in height that has a cylindrical region at the top end (1.37 m diameter and 5 m long) followed by a region with a rectangular cross section that tapers toward the bottom. The cheese curds are fed into the top of the tower, where they are compacted, leading to additional drainage of the whey. Curd compaction is achieved by the combination of vacuum and/or pressure. The cheddared cheese obtained from the towers is then milled and salted. The salted curds are fed into block-forming towers, which have effectively eliminated the hooping and pressing steps in cheese manufacture. These towers are approximately 6 m high and approximately 28×35.5 cm in cross section. The salted curd is further vacuum pressed (and additional whey is drained). The blocks are then cut from the towers and vacuum packaged appropriately.

In addition to the above technologies, the manufacture of pasta filata type cheeses such as mozzarella and provolone involves mechanized equipment in which the cheese curds are transferred to a cooker-stretcher equipped with a twin screw that plasticizes the product at high temperatures (typically achieved with hot water at 82°C [179.6°F] to 85°C [185°F]). The plasticized curd is then molded into desired shapes and passed through a salt brine. The mild shearing action of the twin screws coupled with the high temperature provides the stretching action to the curd, consequently producing the desired texture.

Post Hoop Curd Handling

Non-uniform cooling affects the final cheese quality, especially in cheeses that are packaged in barrels and large blocks. The majority of cheese (mainly cheddar) is now packaged in 500-lb barrels and/or 640-lb blocks instead of the traditional 40-lb blocks due to the ease involved in the post-hoop handling of cheese and therefore enhanced production efficiencies. These barrels and 640-lb blocks cool slower and are therefore associated with non-uniform cooling (Reinbold and Ernstrom, 1988; Barbano, 2001). Non-uniform cooling causes variations in the moisture, salt, and pH within the cheese block (Reinbold and Ernstrom 1988; Reinbold et al., 1992; Barbano, 2001; Carunchia Whetstine et al., 2007).

When these large blocks are cooled from the hooping temperature of approximately 32°C to 35°C (90°F to 95°F) to the ripening temperature below 5°C (41°F), the center of the block cools slower than the surface, leading to higher moisture content on the surface of the block than in the center. The water-holding capacity of caseins depends on temperature. Caseins interact with and hold the water better at lower temperatures. Therefore, during the cooling of large blocks, the water migrates from the center of the block (which is warmer) to the surface of the block (which is colder) (Reinbold and Ernstrom, 1988; Barbano, 2001). In various controlled studies, moisture differentials ranging from 1% to 5.7% have been reported between the surface and the center of a 640-lb block (Reinbold and Ernstrom, 1988; Reinbold et al., 1992; Barbano, 2001; Carunchia Whetstine et al., 2007).

The pH of the block (lower in the center when compared to the surface) also is affected, consequently causing variation in the protein breakdown. This subsequently causes variation in the flavor and texture in the block, thereby affecting the block's consistency (Carunchia Whetstine et al., 2007). The inner regions of the cheese block were found to ripen faster than the outer regions. The inner regions developed a more aged flavor and were firmer and more fracturable, compared to the outer regions. The texture of the outer regions was more cohesive (Carunchia Whetstine, 2007).

This variation in flavor and texture within the same cheese block can prove to be advantageous to food processors in terms of cheese selection as an ingredient. Although presently there is no good way to ensure uniform cooling of large blocks, the use of insulated packaging material (such as plywood) for large blocks has been found to prevent the large temperature differential between the surface and the center, consequently reducing the moisture difference (Reinbold and Ernstrom, 1988).

Packaging Cheese

Cheese packaging has undergone dramatic changes in recent years, with a continuing trend for centralization of cheese packaging for retail cuts. Improvements in cheese packaging materials have enabled consumers to enjoy the finest cheeses of global origin.

Nevertheless, cheese packaging offers unique challenges. Containment, as a function of packaging, protects cheese during ripening and allows it to be presented to the consumer. A properly packaged cheese protects it from moisture loss, chemical degradation, and microbial spoilage, giving it an extended shelf life under the various hazards of the distribution channels. The fact that cheese is a live food adds another dimension to the packaging challenge. Packaging material, in addition to the protective role, must include a definite feature to allow for the uninterrupted cheese fermentation process.

The gas barrier property of the packaging material bears significance in that evolving gases such as CO_2 in most cheeses, especially Swiss varieties, must be expelled from the package. At the same time, oxygen must be kept out to protect the product from fungal growth. More packages are available for reuse than before.

Wax Packaging

Colored cheese waxes have been used in cheese packaging since the late 1930s. Wax coating has been largely replaced by film packaging, but some specialty cheese and artisan cheese makers still prefer wax coating as a distinctive feature.

The waxes coat the surface of cheese or may be combined with tight wrapping with plastic film. Cheese may be wrapped first, followed by dipping in molten paraffin wax. Alternatively, cheese may be waxed prior to film wrapping. Plain paraffin does not possess the tensile strength required for the rigors of modern transportation and handling. Several blends of plain paraffin and microcrystalline waxes are available to prevent cracking of wax coating. Depending upon the toughness of the wax coating, certain elastomers are incorporated to yield peelable coatings. Flexibility is enhanced by using soft wax and a relatively large proportion of elastomer. This property imparts tackiness to the coating, which is ideal for overwrapping with Saran™ film. Other blends are formulated for use in high-moisture cheese or for ripening Swiss and blue types of cheeses.

Wax does not adhere readily to moist cheese surfaces; therefore, a surfactant is added to certain wax blends to facilitate adhesion properties. The color of cheese package is sometimes associated with the cheese variety. For example, bright red is the color of Gouda and Edam cheese. Similarly, black wax has a connotation of aged Cheddar.

Longhorn and some semi-soft cheeses are identified by their yellow wax coating. Other colors available are white, cream, green, blue, cherry red, and brown. Waxes also are available with light transmission varying from clear to opaque. Transparent coating may allow light to be transmitted through the coating, causing oxidation of fat and resulting off flavors in retail cuts of cheese. It also may have a bearing on the shelf life of cheese.

Dyes used for color effect are insoluble. Accordingly, it is imperative to agitate or circulate molten wax to keep the dye in suspension and ensure uniformity of color from batch to batch. The pigment should not be allowed to settle on the heating element or heating surface to avoid overheating and loss of wax. Temperature maintenance in the wax tank is difficult unless the insulating effect of the pigment is controlled by adequate mixing of the dye and the wax.

Two coats of wax are necessary for extended shelf life of cheese. The temperature and dipping time determine the thickness of the wax coating. Pinholes or cracks left by the first coat are covered by the second dip. It is customary to apply the first coat to cheese at a wax temperature of 110°C (230°F) to 135°C (275°F) for 5 to 8 seconds. Following draining and solidification of the wax, the second dip is at 71°C to 82°C (160°F to 180°F) for 3 to 4 seconds. The temperature and time of wax dipping should be standardized for the size and type of cheese in relation to its shelf life requirements. In general, higher temperatures and shorter dipping periods give a thinner coat.

Frequently, a conveyer belt of stainless mesh is used. Cheese cuts or forms are conveyed through a trough containing molten wax for the first dip. The conveyer moves through another trough containing chilled water to congeal the wax coating. Next, the conveyer moves through another trough where the second coating of wax is applied. Finally, the conveyer travels through another chilled water trough to solidify the wax. The waxed cheese may then be wrapped in film, sealed, and shrunk by steam or hot air to give a skintight seal.

Paraffin wax, which displays excellent permeability to CO_2, is useful as a first coating on young cheese. Flexible waxes may be used for a second coating of young and long-hold cheese coated with paraffin wax. Blue cheese held for ripening may be coated with low-temperature flexible wax (containing elastomers) at 66°C to 76°C (151°F to 169°F).

Film Packaging

Film packaging is the most common type of packaging for retail cheese as well as for cheese prepared for ripening. Three methods are widely used: tight wrapping, vacuum gas flushing, and vacuum packaging.

Tight wrapping involves either machine or hand wrapping of plastic films or foils around cheese portions followed by heat sealing. Tight-wrapped cheeses may be dipped in wax either before or after film packaging. The package also may be dipped in sorbate preservative solution. This technique is used for different shapes, sizes, and textures of cheeses. It is labor intensive and time consuming and lacks the shelf life obtained by the flush and vacuum techniques.

The vacuum packaging method uses special films capable of shrinking after heat application. Following evacuation of air from the cheese package, the final seal is accomplished by heat impulse treatment. Cheese for ripening is vacuum packaged. Shrink films are suited for irregularly shaped and soft cheeses. This technique also can include preformed pouches.

The vacuum-gas flush technique is common in retail cheese packaging. Laminated film pouches containing cheese are evacuated and subsequently flushed with CO_2 or nitrogen. Use of CO_2 results in partial shrinking of the bag because the gas reacts with water in the cheese to form carbonic

acid. Accordingly, packages start out loose but become skintight a few days after packaging. The materials generally provide good moisture and gas barrier properties to give excellent shelf life to cheese. The available roll-stock form-fill-seal machinery is fast, versatile, and capable of accommodating a variety of cheese shapes and sizes. One gas flush packaging machine uses roll-stock materials that are formed into bags around the cheese, evacuated, gas flushed, and heat sealed.

The thermoformed cavity method uses reel-fed deep draw materials converted into pouches that are filled with cheese, evacuated, and heat sealed with an additional layer of plastic film that acts as a lid. Shrink film is not generally used. Machines using deep draw forming films are fast and efficient. They give a shelf life comparable to that of gas-flush system. A drawback is that the package exhibits overwrap configuration, which poses difficulty in cartoning and storage. Punctures and leaks result in total package failure due to lack of vacuum.

Another method entails secondary sealing after evacuation of air and shrinking of the film. In this procedure, cheese portions are bagged in shrinkable film, evacuated by the deep draw method, heat sealed, and then passed through a heat chamber. The resulting package is free from ears and excess materials and has an excellent seal because of the double protection afforded by heat sealing and shrinkage. Localized punctures do not cause total package failure.

Hard Cheese Packaging

In hard cheese packaging, process cheese is generally packaged hot into tubs, with wraps open within the molds, and allowed to solidify. Individually wrapped slices are also made by pumping thin sheets that solidify by cooling.

Ripened hard cheeses are sliced, cubed, or grated and packaged under vacuum or inert gas flush. The Hayssen RT horizontal system employs the form-fill-seal method, using a single web of a lamination containing polyester and nylon with polyethylene as the heat sealant (Brody, 2008). Cheese in sliced or block form is delivered to the machine, which conveys it to the continuous flow wrapper, forming a long tube around the contents. Inert gas flows in to displace the air and a long back seam is accomplished. Transverse seams are formed between individual cheese units.

Another system (Multivac) involves the use of a twin web horizontal thermoform-fill-vacuum-gas flush system. The gas barrier base consists of thermoformable film comprised of nylon coated with polyvinylidene chloride (PVDC). This base film is heated and thermoformed in line, creating cavities into which cheese chunks and slices are deposited. Simultaneously, a top web of flexible material is placed on the opening and sealed partially onto the base of the web flanges. The top closure material is made of polyester coated with PVDC and the sealant is comprised of polyethylene. Next, the cavity is evacuated and if desired, gas flushed. The packaging material assumes a collapsed appearance, is given a final seal, and individual packages are separated apart from each other.

Soft Cheese Packaging

Soft cheeses are normally pumped into thermoformed polystyrene or injection-molded polypropylene cups or tubs. The container is then closed with a combination of aluminum foil heat sealed to the flange or friction fit thermoform, with or without a tamper-resistant ring around the rim (Brody, 2008). In certain soft cheeses, the product is pumped into molds and then cut to be overwrapped. Alternatively, the soft cheese is pumped hot into aluminum foil overwraps. Hot packaging tends to reduce the microbial load. Another technique for packaging soft cheese

uses of thermoform-fill-seal machines with polystyrene as the base cup material and aluminum foil lamination as the heat seal closure.

Packaging Films

Several varieties of films are available for retail cheese packaging. These include a vast array of structures based on aluminum foil, polyethylene (PE), polyamide, polyester, and paper. They can be used singly or in combination as laminated structures. They may be purchased as preformed bags or roll stock. Film developed specifically for use in cheese packaging can be obtained from several suppliers.

Basic Film Types

Most tight-wrapped methods use simple, economical materials for films, such as clear plastic wrap—polyvinylidine chloride (PVDC) material of good barrier characteristics. Aluminum foil laminates also are used for some tight-wrapped cheeses.

Gas flush and vacuum packaging systems require laminates. The shortcomings of single-material films can be overcome by combining two or more materials to form laminated structures. The components of laminated films are chosen so that each contributes functionality desirable to the total system. For example, PVDC and nylon are often used as components of laminates; they provide excellent barrier and flexural properties, respectively.

Packages used for gas flushing require materials that must have good machine ability, flex crack resistance, good barrier characteristics, and heat sealability.

Shrink films adhere closely to the cheese surface when heated by hot air or by dipping in hot water at 82°C to 93°C (180°F to 199°F). They are produced by orienting or stretching the film under controlled conditions. PVDC, polyethylene (PE), polyester, polypropylene (PP), polyvinyl chloride (PVC), and several other materials have shrinking attributes. Shrink films for cheese packaging must have good shrink properties, abuse resistance, and seal strength. They generally possess good water and gas barrier attributes for protecting cheese from mold growth, oxidative degradation, and dehydration.

For packaging Swiss and other gas-producing cheeses, differential permeability films are used. These allow CO_2 to escape the package, while preventing oxygen from permeating into the package. This attribute prevents formation of puffed packages due to release of gases by cheese.

Films for use in thermoform machines must be easily heat formed for molding into cavities. Several other films have been made available to accommodate the special needs of relatively unusual cheese varieties.

Basic film requirements include suitability of printing and no transfer of odors or off flavors to cheese. The films must not contain toxic monomers, plasticizers, and inks capable of migrating into the food. Sustainability attributes are beginning to assume a great deal of importance as well. The film must adequately control the head space environment of the packaged cheese to prevent oxidative deterioration, dehydration, and undesirable mold development. The head space environment is influenced by the packaging method used and characteristics related to the particular cheese variety being packaged. Seal integrity, selective barrier properties, and the ability to cling closely to the cheese body are all variables that can be controlled by selection of suitable films.

Packaging for Cheese Ripening

In general, the packaging requirements for ripening blocks of cheese are similar to those for retail portions. Historically, most cheeses for ripening were packaged by bandaging and dressing. Bandaging involved the appli-

cation of a calico or cheesecloth bandage, followed by treatment of the cheese with greased muslin. The structure was either sewn or glued on with flour paste. Dressing the cheese involved the application of wax, oil, or fat. Cheese treated in this manner developed a protective rind as a result of surface dehydration.

Practically all of the cheese for ripening now is packaged in films and foils. Tight-wrapping with certain films has been used for large ripening blocks. Shrink films also can be used. In the case of Swiss type cheese in which gas formation during ripening is considerable, films that absorb CO_2 or have lower barrier properties can be used. Expandable materials also can be employed, but space must be provided to allow for package expansion. Blue cheeses also can be ripened in shrink film bags. However, the cheese wheels must be perforated after packaging to allow access to oxygen, which is necessary for the blue mold development.

Impact of Packaging on the Shelf Life of Cheese

The shelf life of packaged cheese in retail channels depends on several factors. Cheese deterioration at this stage may be related to milk quality, plant sanitation, manufacturing, and packaging procedures including handling and storage conditions. Natural cheeses generally do not contain added preservatives nor are they heat treated to control mold development.

In the case of process cheese, imitation cheese, and cheese analogs, extended shelf life is obtained from pasteurizing heat treatments and application of permissible antimycotic agents. In both natural and process cheese, a suitable package must minimize oxidative deterioration of cheese color and flavor, and protect the cheese from moisture loss as well as from mold growth.

Contrary to the nature of bacteria, most molds thrive in acidic conditions and grow over a wide pH range of 2.0 to 8.5. Many molds can grow well at normal refrigeration temperatures, although the optimum temperature for growth is generally from 25°C to 30°C (77°F to 86°F). Cheese packaged in plastic film does not develop a rind around the periphery, so ample surface moisture and nutrients are available to support mold growth, provided sufficient oxygen is available.

Several popular cheeses are ripened by molds and require oxygen for proper development. Internal mold-ripened varieties (for example, blue, Gorgonzola, Stilton, Roquefort) lose their blue marbling effect after storage in vacuum packages. Nonetheless, many Roquefort-type cheeses are tight-wrapped in aluminum foil or plastic laminates. Hard, thermoformed trays with aluminum foil lids have been used to package crumbled blue cheese varieties.

Internal mold-ripened cheeses (Brie, Camembert) can develop off-flavors if allowed to ripen anaerobically. Different films and foils have been used for packaging these varieties. For example, aluminum foil laminates with certain perforations are used to allow a controlled amount of oxygen gain and yet prevent dehydration. Plastic-lined metal cans also have been used, with cheeses being autoclaved at 2 to 3 psi for several minutes to gain better shelf stability. However, flavor changes may be noticed, depending on the type of metal can used.

For most cheese varieties, undesirable mold growth is the major cause of product deterioration. Vacuum packaging, gas flushing, and tight shrink-wrapping techniques are designed to exclude oxygen in the film package and thus prevent mold development. An oxygen balance sheet involves the balance between oxygen-gain and oxygen-loss factors. Oxygen gain factors are:

1. Enclosed oxygen under the film in wrapping
2. Leakage through defective seals, in overlaps, or at end folds

3. Leakage through punctures or chafed areas of the film
4. Permeation of oxygen through the film

The oxygen-loss factors are absorption of oxygen by cheese (bacterial action, reducing systems) and utilization of oxygen by mold growth.

Entrapped Oxygen

Shrink films generally provide close film contact with the cheese body, resulting in minimal oxygen entrapment. Very soft or non-uniform cheeses (mozzarella, pizza), in which entrapped oxygen may be a problem due to irregularities of the cheese, are commonly packaged in shrink films. Process and artificial cheeses can be hot-poured directly into packaging materials. This also provides close film contact with the cheese. Sliced processed cheese may be rolled off cold drums, sliced, and automatically packaged. Alternatively, it may be hot-packaged by extrusion into a tube and then sealed, compressed into a flat strip, cooled, cut, and overwrapped in a polyester film.

The concentration of entrapped oxygen can decrease within a package due to evolution of gases during cheese ripening. It has been observed that oxygen concentration declines rapidly after the first few days of storage, whereas CO_2 concentration increases in certain types of cheeses. In general, no mold growth is observed when the oxygen concentration in the gas underneath the wrapper is less than 2% and that of CO_2 is greater than 27%. Most cheeses produce a small amount of CO_2; the extent of its evolution depends on the type of microorganisms present. However, Swiss-type cheeses produce extensive CO_2 during ripening and storage, so mold development due to entrapped oxygen is unlikely.

Entrapped oxygen also may be utilized by the cheese. Freshly packed cheddar cheese possesses considerable reducing power and is able to utilize entrapped oxygen, making it unavailable for mold development. The reductive power may be elevated at higher temperatures due to increased culture activity. Because older cheeses contain lower microbial populations, they do not have much capacity to utilize oxygen present in the cheese package. Mold growth appears to increase at lower temperatures for relatively young cheeses. Consequently, cheese should be held at a temperature higher than refrigeration for one or two days immediately after packaging to utilize entrapped oxygen.

Gas Barrier Properties

It is critical to control the amount of oxygen permeating through the material or seal of the cheese package. Some literature suggest an oxygen barrier for cheese of not less than 5 cc/100 in^2/24 hours at 22.8°C (73°F) and 50% relative humidity. Cheese variety and conditions of storage can affect the necessary barrier requirements, and a barrier of less than 1 cc/100 in^2/24 hours may be necessary in some cases.

As indicated earlier, the extensive gas evolution of Emmental (Swiss) varieties may inhibit mold development. Films for use in these cheeses often have relaxed barrier attributes, which allows diffusion of CO_2 from the package and prevents package rupture. The hydrophilic properties should be considered when selecting a film. Storage at high relative humidity or direct contact between a hydrophilic material and cheese portion can adversely affect the water vapor and gas barriers. Decreasing temperature generally improves the barrier characteristics of films; the extent of change depends on the material.

Seal Leaks

Seal leaks are probably the most common and important factors leading to mold development in film-packaged cheese. They have

been cited as the major cause of decreased shelf life in tight-wrapped cheese compared to cheese packaged by gas-flush or vacuum techniques. Wax treatment after tight wrapping, or the use of sorbates, may improve package and product stability.

Heat sealing is commonly used for shrink bag, vacuum, and gas-flush systems. Extensive leaking of the heat seal can cause package failure in gas-flush and evacuation methods. A seal integrity test should be conducted at regular intervals on the cheese packaging line to ensure proper settings on the packaging equipment.

Oxidation

Oxidation typically begins on the exposed food portion. It depends on the presence of oxygen, and is catalyzed by light, metals, enzymes, heat, and ioninizing radiation or ultraviolet light. Oxidation problems can occur in consumer-sized packages because of their relatively large surface area and exposure to fluorescent light while sitting in display cases at the grocery store. The sensitivity of the annatto (Bixin) pigment to oxidative deterioration is ascribed to its highly double-bonded structure. Storage temperature influences oxidative degradation of cheddar cheese. Temperature may influence both oxidation kinetics and microbial activity. Young and active cultures may reduce or eliminate necessary oxygen despite the positive effects on oxidative kinetics. Mold growth and fat oxidation seldom occur together. It is postulated that mold development utilizes oxygen within the package. A higher incidence of discoloration of cheddar cheese has been observed at lower temperatures. Storage at 2°C (36°F) is reported to increases peroxide values (indicator of oxidation) of vacuum-packed cheddar as compared to storage at 13°C (55°F). This has been attributed to greater reducing power at the elevated temperature as a result of increased bacterial activity.

Photo-oxidation is distinguished from spontaneous auto-oxidation in several ways. Light energy can catalyze oxidative initiation reactions by stimulating the production of free radicals or by causing the formation of singlet oxygen. After initiation, light can catalytically degrade hydroperoxides by photolysis. Ultraviolet light and some wavelengths of the visible spectrum cause light-induced off flavors in dairy products. Green, red, blue, and brown materials have been used to prevent light induced off flavors.

Dehydration

Many cheeses may be categorized as intermediate-moisture food with high water activity. Without packaging, the cheese develops a rind due to water evaporation from the surface as was practiced in the industry in the past. The rind retards, but does not stop, further loss of moisture from the cheese. A water barrier of not less than 1 g/100 in^2/24 hours for cheese packaging materials in necessary. Most modern cheese packaging films and foils provide a good moisture barrier. Except in cases of extensive seal leaking or pinholing, film-packaged cheese generally does not lose more than 0.1% moisture.

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Chapter 11

Manufacturing Outlines and Applications of Selected Cheese Varieties

Ramesh C. Chandan and Rohit Kapoor

Introduction

The term "cheese" generally refers to conversion of vital milk constituents from fluid to semi-solid or solid form. A crucial step in cheese making is the coagulation of milk, resulting in curd formation. This facilitates consolidation of milk proteins and fat along with the important mineral and vitamin fractions in solid form. This chapter discusses basic manufacturing procedures for selected cheese varieties, including selected natural cheeses varieties, process cheese and process cheese products, and cheese analogs. Data on cheese markets and trends in the United States are also summarized.

Cheese consumption in the United States continues to increase. The total consumption in 2007 and 2008 was 33.2 and 32.5 lbs per capita, respectively (IDFA, 2009). The most popular individual cheese variety in 2008 was mozzarella at 10.7 lbs per capita, followed by cheddar cheese at 10 lbs per capita. Per capita consumption of all Italian varieties grew to 14.1 lbs. The supermarket sales of cheddar cheese in 2008 totaled 563 million lbs, followed by processed American cheese (420 million lbs) and mozzarella cheese (285.6 million lbs).

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Natural Cheeses

Manufacturing Outline for Natural Cheeses

The principles of cheese making are discussed in Chapter 10. The general ingredients in cheese manufacture are reviewed below.

Dairy ingredients include milk, nonfat milk, or cream, used alone or in combination. Milk is defined by the Code of Federal Regulations (CFR, 2009a) as the lacteal secretion, practically free from colostrum, obtained by the complete milking of one or more healthy cows, which may be clarified, and fat may be adjusted by separating part of the fat. Concentrated milk, reconstituted milk, and dry whole milk may be also be used. Nonfat milk solids refers to skim milk, concentrated skim milk, reconstituted skim milk, and nonfat dry milk. Cream is synonymous with cream, reconstituted cream, dry cream, and plastic cream. Water, in a sufficient quantity to reconstitute concentrated and dry forms, may be added.

Pasteurized dairy ingredient means that every particle of such ingredient shall have been heated in properly operated equipment to 63°C (145°F) or 72°C (161°F) and held continuously at or above that temperature for 30 minutes or 15 seconds, respectively. Pasteurization of milk is credited with effective control of foodborne diseases and assurance of public health. Properly pasteurized

milk and other dairy products show negative phosphatase tests and are considered safe from a public health standpoint. If pasteurized milk is used for cheese making, it must be heat treated at a minimum of 62.8°C (145°F) for 30 minutes or 71.7°C (161°F) for 15 seconds. Zero activity of alkaline phosphatase indicates proper and legal pasteurization. However, for cheese made with legally pasteurized milk, positive residual phosphatase activity is observed, depending on the type of cheese. Most cheese varieties show positive phosphatase activity measured as μg of phenol equivalent/g of cheese. This activity is ascribed to phosphatase activity generated by the culture used and ripening organisms. The Food and Drug Administration has recognized this residual activity. If the phosphatase test shows the phenol equivalent value of more than 12 μg/g of cheese, it is considered as violative. Additional details for all of the ingredients used in cheese making, including their functional properties, are discussed in Chapter 10.

Starters comprised of harmless cultures are used for acid and flavor production during cheese making and curing. They are discussed here in relation to specific cheese processes.

Coagulants, or clotting enzymes, include rennet and/or other clotting enzymes of animal, plant, or microbial origin.

Coagulation aids, i.e., calcium chloride in an amount not more than 0.02% (calculated as anhydrous calcium chloride) of the weight of the dairy ingredients, can be used.

Ripening aids, enzymes of animal, plant, or microbial origin, aid in curing or flavor development. The level of such enzymes cannot exceed 0.1% of weight of the milk used.

Cheese colors may be used to give characteristic color to certain cheeses and to give light cream color to cheeses made from winter milk.

Antimycotic agents are optional mold-inhibiting ingredients consisting of sorbic acid, potassium sorbate, sodium sorbate, or any combination of two or more of these, in an amount not to exceed 0.3% by weight, calculated as sorbic acid. They are present in slices or cuts of most cheeses in consumer-sized packages.

Hydrogen peroxide may be used in lieu of heat treatment, followed by a sufficient quantity of catalase preparation to eliminate the hydrogen peroxide. The weight of the hydrogen peroxide shall not exceed 0.05% of the weight of the dairy ingredients and the weight of the catalase shall not exceed 20 ppm of the weight of dairy ingredients treated.

Other ingredients specific for some cheese varieties are discussed in the production methodologies. For a discussion of the role and function of various ingredients involved in cheese making, see Chapter 10.

American Cheese Group

The American cheese group includes cheddar, colby, and washed/granular/stirred curd cheese and Monterrey Jack cheese. Table 11.1 shows the proximate composition of American types of cheese.

Cheddar Cheese

Cheddar cheese is a firm and hard cheese. The U.S. Code of Federal Regulations requires cheddar cheese to contain a minimum milk fat content of 50% by weight of the solids, and the maximum moisture content is specified at 39% by weight (CFR, 2009a).

Cheddar cheese for manufacturing is another variant of cheddar cheese. It conforms to the definition and standard of identity for Cheddar cheese except that the milk is not pasteurized and ripening is not required. This product is made for manufacturing pasteurized process cheese and allied products. Low-sodium cheddar cheese contains not more than 96 mg sodium/lb of finished product. Reduced-fat cheddar cheese contains 19.2% to 22.9% fat and 49% moisture.

Table 11.1. Typical chemical composition of American cheese varieties.

Cheese	pH	% Moisture	% Fat	% Fat in dry matter	% Protein	% Salt	% Lactose
Cheddar	5.4	36.7	33.1	52.4	24.9	1.6	1.3
Colby	5.2	38.3	32.1	52.0	23.8	1.5	2.6
Washed curd/ soaked curd	5.2	41.0	31.0	52.5	23.9	1.4	2.6
Granular/ stirred curd	5.3	38.0	32.0	51.6	24.1	1.4	2.5

Adapted from Nath (1993), Fox et al. (2000a), CFR (2009a)

Cheddar Cheese Process: An outline for the manufacture of cheddar is given in Figure 11.1. Milk is standardized to protein/fat (P/F) ratio of 0.91/1.0 or casein/fat ratio of 0.7/1.0 and transferred to a cheese vat at 31°C (88°F). The mesophilic starter containing *Lactococcus lactis* subsp. *lactis/cremoris* is added at the rate of 1% to 2%. When the culture growth is indicated by pH drop of 0.05 pH unit (increase of 0.01% titratable acidity; TA), optional cheese color may be added at the rate of 70 ml/1,000 kg of milk. For white cheddar, no color is added. Both white and orange-yellow cheddar (color added) are produced in the United States. Rennet is added to the vat at the rate of 190 ml/1,000 kg of milk after diluting with 10 volumes of water.

When the curd is firm enough (usually after 25 minutes), it is cut using 0.95-cm (3/8-inch) knives. After 5 minutes, the curd is agitated very gently for 10 minutes. The vat contents are cooked at the rate of 1°C/5 minutes to reach 39°C (102°F) in 30 minutes. If the whey has a higher pH at cutting, cooking is extended to 60 minutes. Cooking continues until the whey pH drops to 6.2 to 6.3. It may take 75 minutes to achieve the acidity. The curd should shrink to about half of its observed size before cooking. It should be firm with no soft or mushy center. The curd should not stick together when pressed in a hand.

At this point, the cheddaring process starts by removing 50% to 70% of the whey. The curd is continuously stirred and the remaining whey continues to drain to dry out the curd. The curd is piled 4 to 5 inches deep along the sides of the vat for matting/ congealing. Whey drains slowly through the trench in the middle. The front edges of the matted curd are cut, layered on the slabs, and cut further into strips and piled again. The temperature of the curd is maintained at 30°C to 35°C (86°F to 95°F). The slabs are turned repeatedly at 5- to 15-minute intervals.

When the pH of the whey drops to 5.3 to 5.4, the slabs are milled. At this stage, the curd slab should feel reminiscent of chicken breast. Milling involves running the slabs through a milling machine and cutting them into small finger-size curds. After milling, the curds are stirred vigorously to avoid matting. At this stage the curds should have round, smooth edges. The curd temperature should be maintained at 27°C to 32°C (81°F to 90°F). After 15 to 30 minutes, the curd is sprinkled with salt; the amount of salt varies from 2 to 3 kg/1,000 kg of milk. Salting results in more whey drainage, and the more moist the curd, the more salt it needs. The target salt content of cheese is 1.7% (w/w).

Next, a disposable plastic liner is inserted into a 20-lb hoop and 22 lbs of curd are weighed into the stainless steel hoops. The lid is placed on the top prior to pressing. The hoops are lined up in the press and pressed for 12 to 18 hours at 10 to 20 lbs/ square inch^2 (75 kPa). The pressing time is much shorter in automated systems. After pressing, the blocks are removed from the hoops, vacuum packed, and transferred to the ripening room.

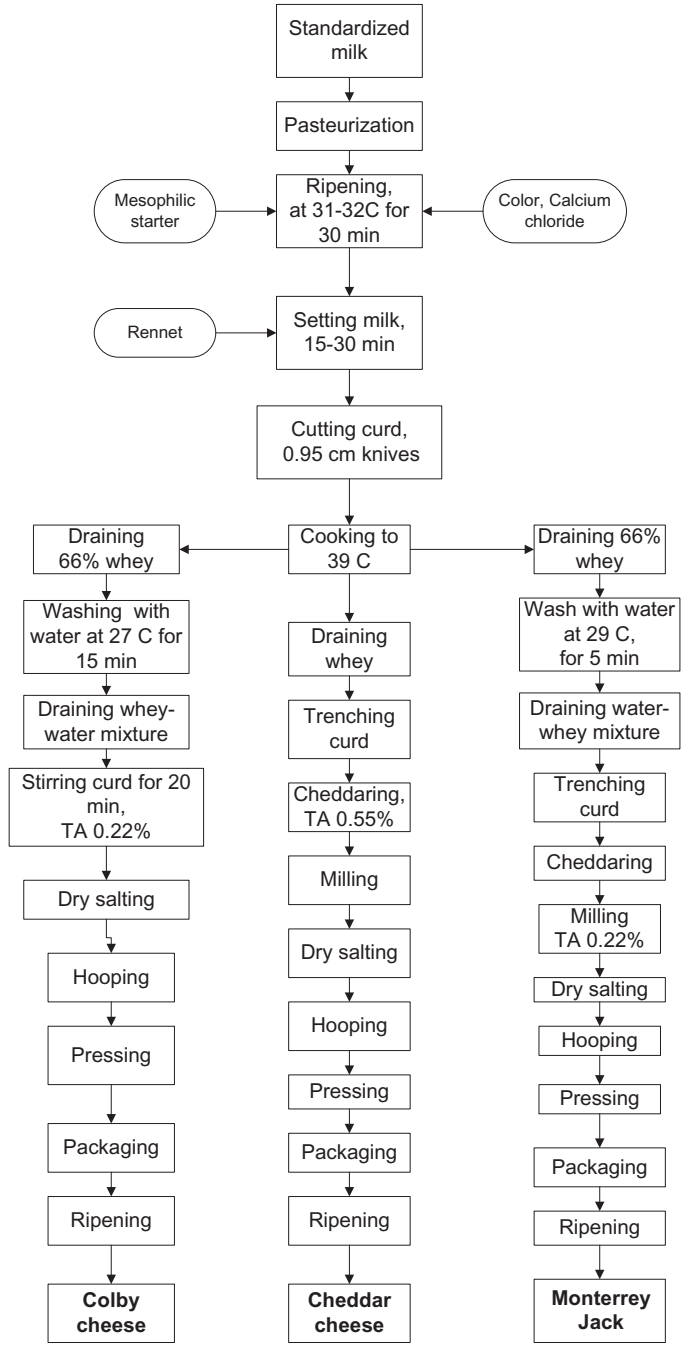

Figure 11.1. Flow sheet diagram for manufacturing cheddar, colby, and Monterrey Jack cheeses.

The ripening temperature varies from 5°C to 8°C (31°F to 46°F) for slow ripening or 10°C to 16°C (50°F to 61°F) for fast ripening. Slow ripening leads to better flavor control. The ripening period may vary from three to nine months or even longer to obtain a sharper flavor. The cheddar cheese standards are a minimum of 50% fat in dry matter and a maximum of 39% moisture. However, for long-hold cheddar, the moisture should not exceed 36%. For short-hold cheddar, the moisture content ranges from 37% to 39%. Cheddar cheese yield is generally around 9.5 to 10 lbs/100 lbs of milk.

Colby Cheese

Colby cheese is a washed-curd cheese and is not as hard as cheddar cheese. It contains not more than 40% of moisture, and its solids contain not less than 50% of milk fat. The color of colby cheese is generally dark yellow to orange, which is achieved by adding cheese color to milk. "Colby cheese for manufacturing" conforms to the definition and standard of identity prescribed for colby cheese except the milk need not be pasteurized and ripening is not required. It is generally used in the manufacture of process cheese products. Low-sodium colby cheese is made with salt substitutes and contains not more than 96 mg sodium/lb of cheese.

Colby Cheese Process. Figure 11.1 shows the basic steps in colby cheese manufacture. Colby cheese manufacture resembles that of cheddar cheese minus the cheddaring step. Instead, the curd is washed in water to remove lactic acid and lactose. The cheese has more moisture (40%) and less fat (29%) than Cheddar cheese. Its texture is more open, its body is softer, and because of the higher moisture, it ripens more quickly than cheddar.

Milk is standardized to a P/F ratio of 0.96, pasteurized, and transferred to a cheese vat at 31°C (88°F). The starter, consisting of *Lactococcus lactis* subsp. *lactis/cremoris*, is mixed into the milk. After approximately 60 minutes, when the TA increases by 0.01%, cheese color is added at the rate of 70 ml/1,000 kg of milk and thoroughly blended. Generally, 1 volume of the color is diluted with 20 volumes of water prior to addition. Then, diluted (1 volume of rennet and 10 volumes of water) 190 ml of rennet/1,000 kg of milk is added and the vat is allowed to set under quiescent conditions.

After the curd has achieved firmness, it is cut using 0.95-cm (3/8-inch) knives. The agitators are run at slow speed and cooking of curd should start after 15 minutes by raising the temperature to 39°C (102°F) in 30 minutes. The cooking temperature should increase slowly at first, at the rate of 1°C/5 minutes. When the temperature is 39°C (102°F), the temperature is maintained for the acidity to develop. It may be up to 2 hours from cutting time when the pH of whey drops down to 6.2 to 6.3.

At this point, the whey is drained until the curd surface is visible. Enough wash water at 15°C (59°F) is added to bring the temperature of the curd-water mixture to 26°C (79°F). A curd table may be used for washing the curd. The amount of water required ranges from 7% to 14%. If the temperature is less than 15°C (59°F), less water is used. Cold wash water leaves more moisture in the curd and vice versa. The curd is washed for 15 minutes and drained. The washed curd is trenched and salted at the rate of 2 kg salt/1,000 kg of milk. After 15 minutes of agitation, the curd is transferred to 20-lb hoops and pressed at 10 to 20 psi (69 to 138 kPa) for 16 to 18 hours. The cheese blocks are removed from the hoops and vacuum packaged in plastic film, sealed, and ripened at 7°C to 13°C (45°F to 55°F) for one to three months. Cheese yield is around 10 to 11 kg/100 kg of milk.

Monterrey Jack Cheese

Monterrey Jack is a semi-soft variety of cheese. It contains at least 50% fat in dry

matter and no more than 44% moisture by weight. It is a mild flavored cheese made by a process similar to that of colby cheese. A variant called Jack cheese contains higher moisture (at least 44% and no more than 50% moisture by weight).

Figure 11.1 also illustrates the manufacturing procedure for Monterrey Jack cheese. Milk is pasteurized and inoculated with 1% starter consisting of *Lactococcus lactis* subsp. *lactis/cremoris*. Rennet may be added right after the culture is added unless it is intended to develop high acidity at draining. The set curd is cut with 0.95-cm (3/8-inch) or 0.62-cm (1/4-inch) knives, depending upon the desired moisture in the cheese. The curd is stirred and cooked to 37°C to 40°C (98°F to 104°F) in a period of 30 minutes. The rate of heating is similar to that used for cheddar cheese manufacture.

After cooking, the curd and whey are stirred for additional 60 to 90 minutes until the curd is firm. The TA should increase by 0.02% over the acidity at the cutting stage. The whey is drained to the level of 1 inch above the curd surface. Cold water is added to bring the temperature of the curd-and-whey-water mixture to 30°C (86°F). The temperature should be lower for higher moisture cheese, and vice versa. The mixture is stirred for another 5 minutes and the whey is totally drained. Occasional stirring is necessary during draining to avoid matting. After 30 minutes, the curd is salted with 2.5 to 2.7 lbs of dry salt/100 lbs curd. Stirring is continued until the hoops are filled. The hoops are lined with fabric and pressed to form 20- to 40-lb blocks. The blocks are vacuum- packaged in plastic films and ripened for three to six weeks.

Granular and Stirred Curd Cheese

Granular and stirred curd cheese has a minimum fat content of 50% in dry matter and maximum of 39% moisture. The curd is produced in identical manner as that of cheddar cheese, except that after removal of part of the whey it is not allowed to mat. It is then heated and stirred in the remaining whey. After draining, the curd is salted, hooped, and pressed. It is generally used in the manufacture of process cheeses.

Washed Curd Cheese

Washed curd or soaked curd cheese contains a minimum of 50% fat in dry matter and maximum moisture of 42%. The ingredients for manufacture are identical to those for cheddar and colby cheeses. For long-hold washed curd cheese, the moisture should not exceed 39%. The process is identical to that of cheddar, except that the TA is only 0.37% to 0.40% at the whey drainage stage. Following whey removal, the curd is cut and soaked in cold water. The addition of water and stirring cools the curd, and water displaces some proportion of the whey from the curd. The required moisture content of 41% is facilitated by cooling of the curd to 30°C to 31°C (86°F to 88°F). The curd is then drained, salted, stirred, and pressed in hoops. In the manufacture of "washed curd cheese for manufacturing" there is no requirement for use of pasteurized milk. This product is used for manufacturing process cheese products.

Quality Issues Related to Cheddar Cheese

Appearance, Body and Texture Issues: In addition to flavor, color, appearance, body, and texture play a critical role in the overall quality and grade of cheese. Table 11.2 illustrates issues related to the color and appearance of cheddar cheese.

For judging true flavor, body, and textural characteristics of cheese, it is necessary to temper the sample of cheese to 10°C to 15.6°C (50°F to 60°F). A cheese trier, a curved, double-edged knife, is useful for sampling large blocks of cheese. The trier is inserted into the cheese, rotated one-half turn, and pulled out to retrieve a long piece of cheese sample called the plug. The plug is then examined for aroma, body, and texture.

Table 11.2. Problems related to the color and appearance of cheddar cheese.

Defect	Probable cause	Remedial measure
Acid-cut, bleached/faded, dull looking portions or on the entire surface of the cheese	Excessive acid development in the whey or at the packing stage Non-uniform moisture distribution in the cheese	Watch acid development carefully Take precautions to ensure consistent and uniform moisture retention in the curd
Mottled appearance, irregularly shaped light and dark areas on the cheese surface	Combining curds of different colors, batches, or moisture contents Uneven acid development in curd Growth of yeast and bacteria accompanied by typical fruity flavor and pasty body H_2O_2 production by microorganisms	Avoid mixing starter after color addition Strain the starter before adding to the vat Try to cut curd into uniform size particles Handle curd carefully to avoid drying during the matting and cheddaring steps
Seamy, showing light-colored lines around curd pieces; cracked cheese in the extreme form	Exudation of fat in curd pieces due to excessive forking Warmer temperature Lack of dissolution of salt	Press the curd at 29 to 32°C (85° to 90°F) Allow all of the salt to dissolve completely Avoid too much forking of the curd Wash greasy curd at 32°C (90°F) and drip dry the curd immediately.
White specks or granules or smeared with white powder material	Generally present in aged cheese. Derived from crystallization of calcium lactate	Ripen at higher temperature/shorter curing period Avoid exposure to UV light
Moldy appearance	Mold growth on the surface	Ensure tight seals on cheese packages Avoid oxygen in the package by vacuum/flushing with CO_2/N_2 gas

An ideal quality of cheese body and texture is indicated by a waxy and smooth-sided plug that bends fairly well and does not snap but breaks slowly upon bending. Flavor, color, body, and texture are intimately connected with the fermentation pattern. Chemical changes in protein and lipids lead to their breakdown products, which are chemically simpler and more volatile in nature. Accordingly, specific bacteriological and biochemical transformations must be fostered and controlled in cheese during manufacturing and curing to create consistently good-quality cheese. Table 11.3 gives information on defects in body and texture.

Table 11.3. Body and texture defects in cheddar cheese.

Defect	Probable cause	Remedial measure
Corky, dry, and hard	Lack of acid development Low fat in cheese Overcooked cheese curd Low moisture retention in the cheese curd Excessive salt levels	Follow standard production procedures
Crumbly, mealy, and short body	Excessive acid production and low moisture retention in the cheese	Avoid ripening at high temperatures Control acid development and moisture level in the cheese
Rubbery or curdy	Lack of curing conditions	Optimize ripening time and temperature
Pasty, sticky, or wet	Excessive acid development Excessive moisture content,.	Control acid development in relation to time and temperature parameters,.
Weak/soft	Fat content too high Moisture content too high	Standardize the fat in cheese milk Cook the curd to desirable firmness (higher temperature, longer period) Avoid piling curd slabs too high or too soon while cheddaring the curd

Frequently, defects in color, body, and texture are concomitantly noticed with flavor problems in cheese. In this regard, open texture or holes in cheese may pose problems ranging from minor to serious. These holes may be mechanical or biological in origin. The size, appearance, and shape of the hole may give a clue to the problem. Mechanical holes are recognized by their irregular size and angular shape, and they have a dull appearance. This defect arises from improper knitting of curd during pressing. It can be traced back to unfavorable conditions during acid production, cooking, matting, greasy curd surface resulting from improper temperature, undissolved salt, and inadequate pressing procedure. Because this defect is basically mechanical in nature, no flavor problems are associated with it. Consequently, it is not considered as a serious defect.

Open texture of biological origin may be classified as follows:

- Fish eyes, ascribed to yeast fermentation, are characterized by a round, glossy, and gas-induced open texture. Excessive gas generation by yeast growth leads to spongy or honeycomb-like body, eventually flattening to give long narrow slits.
- Small pinholes are identified as being fairly uniform and in large numbers. They are derived from the production of gas as a result of undesirable bacteria-like coliforms within the cheese body. Extensive growth of the bacteria (1 million CFU/g) causes a spongy or bloated condition with an accompanying fruity flavor defect.
- Large, uniformly distributed gas holes resemble Swiss cheese eyes. If the holes are caused by the growth of *Propionibacterium shermanii*, a definite sweet and nutlike flavor accompanies the appearance of such holes.
- Slit or fissure formation is recognized by a narrow and long cut in the cheese. It is also microbial in origin in that gas formation prevents complete welding of curd surfaces.

Openness in texture due to gassiness may be ascribed to coliforms if it manifests during the early stages of ripening. However, late blowing during ripening is generally due to gas production by Clostridia. In addition, starters may contain gas-producing culture. Cheese-making conditions leading to excessive lactose in the curd lead to gas production by yeasts and other microorganisms. Proper acid development, moisture control, and desirable salt levels are the factors necessary for suppressing gas producers in cheese.

Flavor Issues: The nature and intensity of flavor are considered to be critical factors in consumer acceptability. In addition, manufacturers of process cheese are concerned with the flavor, body, and texture of various lots of natural cheese, which are graded on flavor, body, and texture so that after blending, the desired characteristics are built into the finished process cheese. To maximize market acceptability and economic returns, cheese manufacturing operations engaged in natural cheese varieties must maintain optimum flavor quality. Manufacturing variables usually controllable by a cheese maker contribute significantly to fluctuations in the quality of cheese flavor. Table 11.4 lists flavor defects, causes, and actions to correct the problems.

Freshly pressed cheese displays little or no typical flavor commonly associated with cheddar or colby cheeses. Presence of nontypical flavors at this point indicate quality issues with cheese milk, starter inactivity, or contamination, and probable inconsistencies in the cheese process. Strictly following standard procedures to achieve a consistent rate of acid development, cooking temperature and time, curd matting technique, and salting regimes ensures minimal variation in cheese flavor quality.

A reasonable constant retention of moisture, fat, casein, and minerals in cheese curd should ensure a uniform fermentation medium for ripening organisms. Moisture control factors include:

Table 11.4. Flavor problems in cheddar cheese.

Flavor defect observed	Probable cause	Remedial measure
Bitter flavor	Excessive moisture Low salt level Proteolytic strains of starters and/or contaminating microflora Excessive acidity Poor milk quality and/or sanitary conditions	Use less starter Ripen for shorter time and/or at lower temperature Check salt level and salting technique Check starter for purity/suitability Improve cleaning and sanitizing practices Control acid and rate of acid production
Sour flavor	Excessive acidity Excessive moisture Too high starter level High-acid milk used Improper expulsion of whey from the curd Low salt level	Use less starter Shorten the ripening period Follow standard procedures for the cutting, cooking, and salting steps
Fruity/yeasty	Low acidity Excessive moisture Dirty equipment Poor-quality milk	Improve sanitation Check and improve water quality Follow standard procedure for cheese making and equipment cleaning Check salting procedure
Rancid/soapy	Milk lipases activated by improper milk production and handling practices Microbial lipases from contaminating microflora Late lactation milk or accidental homogenization of milk	Check cheese milk for rancid flavor Avoid excessive agitation, foaming, and temperature fluctuations of raw milk Avoid microbial contamination of milk and cheese by improving sanitation
Weak/no flavor	Lack of acid production Low-fat milk used for cheese making Excessively high cooking temperature Too-low ripening temperature/ too-short ripening period	Check starter activity Increase starter level Check curing temperature Extend curing period Follow standard procedure for fat standardization in milk and cheese making
Musty/moldy	Extensive mold growth on cheese surface	Seal cheese blocks/barrels to eliminate oxygen entry
Miscellaneous off flavors: barny, feed, malty, onion, weed	Usually associated with milk production (feed and physiological condition of cows)	Avoid milk with these flavors Vacuum pasteurize milk prior to cheese making to volatilize off most of these flavors associated with raw milk

- Size of wire knives, which determines the surface area of curd particles. A smaller size favors reduction of curd moisture.
- Higher cooking temperature and extended period of stirring curd in whey (especially at higher acidity) favor loss of curd moisture.
- Higher acidity during the cheddaring and milling steps favors lower curd moisture.
- Temperature fluctuations during cheddaring influence both acid production and moisture retention in cheese. Keeping the curd warmer leads to drier curd. Subsequent to cheese pressing, the rate of flavor development can be controlled by ripening temperature and curing period. In general, under comparable ripening conditions, development of both desirable flavor and undesirable taints are related to the moisture level and salt content of cheese.
- Strain composition and activity of the starter constitute effective criteria to

develop acidity and to manipulate conditions for effecting a desirable moisture level. Proper rate of acid development leads further to suppressions of many pathogenic organisms, creating cheese that is considered safe for public consumption. In the absence of proper acidity, gas-forming and unwanted acid producers could invade and prosper in cheese, giving rise to serious flavor problems.

Italian Cheese Varieties

Total cheese production in the United States in 2008 was 9.9 billion lbs (IDFA, 2009). Italian cheeses, particularly mozzarella cheese, are the most popular cheeses, accounting for production volume of 4.158 billion lbs in 2008. Italian cheeses accounted for nearly 42% of the total cheese produced.

Mozzarella cheese dominated and accounted for 77.9% of the total Italian cheeses, followed by provolone at 7.9% and ricotta at 6.2%. In contrast, the American cheese group (cheddar, colby, monterrey, and jack) registered 4.071 billion lbs and constituted 40.9% of the total cheese production.

Italian cheeses may be classified according to their hardness. Figure 11.2 shows their classification according to Reinbold (1963).

The composition of Italian cheeses is shown in Table 11.5. In view of their commercial importance in the United States, this

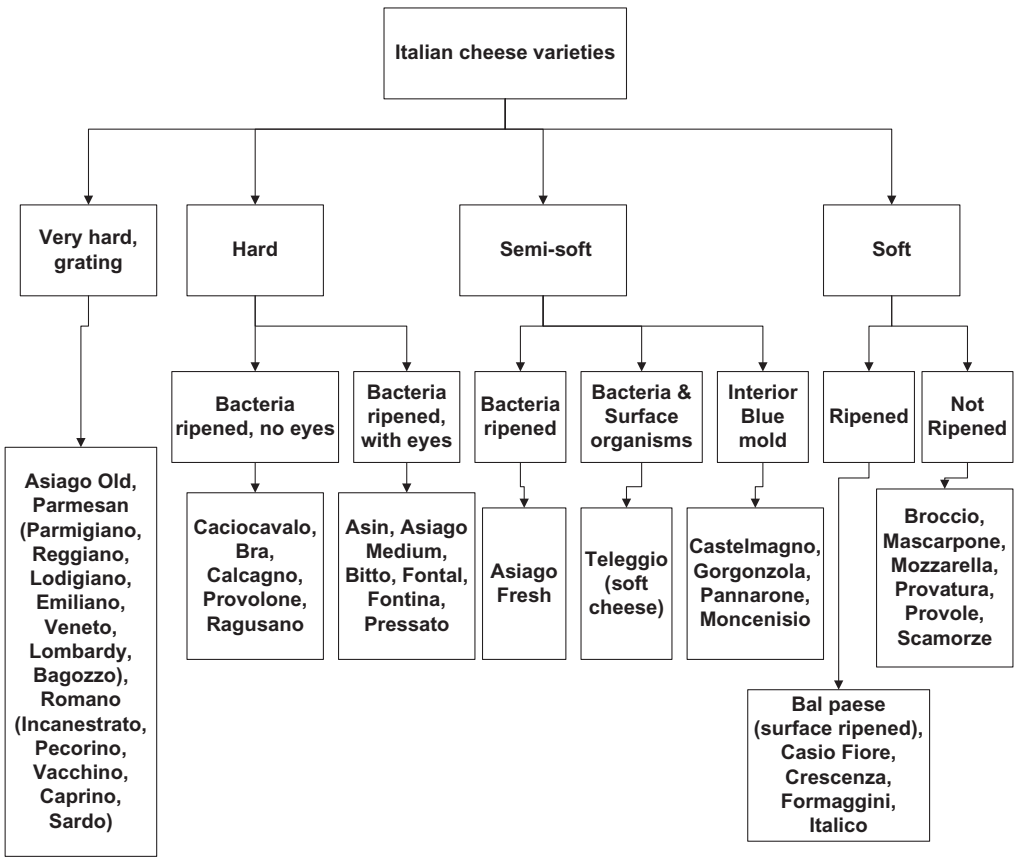

Figure 11.2. Italian cheese varieties. Adapted from Reinbold (1963).

Table 11.5. Approximate composition of some Italian cheeses.

Cheese	pH	% Moisture	% Fat	% Fat in dry matter	% Protein	% Salt	% Lactose
Mozzarella, whole milk	5.2	54.1	21.6	45.1	19.4	1.8	2.2
Mozzarella, part skim	5.2	55.0	17.9	40.3	21.6	1.6	2.3
Mozzarella, low moisture	5.2	49.5	23.9	47.5	22.8	1.5	2.2
Mozzarella, part skim, low moisture	5.2	48.5	21.0	37.9	26.3	1.4	2.2
Lite mozzarella	5.2	57	10.5	24.4	15.5	1.4	2.4
Provolone	5.4	42.5	26.6	46.1	25.0	3.0	2.1
Parmesan	5.4	29.2	25.8	36.5	35.7	2.2	3.2
Romano	5.4	30.9	26.9	39.0	31.8	4.8	2.6

Adapted from Kindstedt (1993), Fox et al. (2000a), Nath (1993)

section discusses mozzarella, provolone, parmesan, and Romano cheeses further.

Mozzarella Cheese

Mozzarella and pizza cheese belong to the pasta filata group, in which the curd is characteristically melted, kneaded and stretched, and molded during its manufacture. This treatment imparts the desired melting and stretching attributes. The variants of mozzarella cheese in the industry are as follows:

- Mozzarella cheese must contain minimum milk fat of 45% (fat in dry matter basis, FDM). The moisture content must be 52% to 60% by weight.
- Low-moisture mozzarella cheese contains the same fat content as mozzarella cheese (minimum milk fat content of 45% FDM); however, the moisture content is lower than that of mozzarella cheese (45% to 52% by weight).
- Part-skim mozzarella cheese contains lower fat content than mozzarella cheese (30% to 45% FDM); however, the moisture content is identical to that of mozzarella cheese (52% to 60%).
- Low-moisture, part-skim mozzarella cheese contains the same fat content as part-skim mozzarella cheese (30% to 45% FDM); however, the moisture content is identical to that of low-moisture mozzarella cheese (45% to 52%).
- Reduced-fat mozzarella cheese contains 7.5% to 9.5% FDM and moisture content of 53% to 55%. Its pH is 5.2 to 5.3 and its salt level is 0.8% to 1.2%.
- Low-fat mozzarella contains 5.5% to 9.5% fat and 58% moisture. Its pH is 5.3 and its salt level is 0.6% to 1.0%.

Mozzarella Cheese Process: A flow chart for the process is shown in Figure 11.3. Both the traditional culture and direct acidification processes are shown.

The traditional procedure is as follows. Milk is standardized to a fat content of 1.6% to 3%, depending on the fat content desired in mozzarella cheese or its variants. After pasteurization, milk is pumped into a vat at 31.1°C to 33.3°C (88°F to 92°F). The starter, consisting of mesophilic culture *Lactococcus lactis* subsp. *lactis/cremoris,* is added at the 0.1% to 0.2% level. In addition, thermophilic culture (*Streptoccus themophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus)* may also be used at the 0.1% level. Some processors use yogurt culture exclusively at the 0.5% level. When the TA increases by 0.02% in about 30 minutes, rennet (190 ml/1,000 kg milk, diluted 10 times with water) is added. The milk sets in about 30 minutes.

The coagulum is cut with 0.95-cm (3/8-inch) or 1.27-cm (1/2-inch) knives. The TA of whey should be around 0.125% at this stage. The curd is allowed to stand undisturbed for

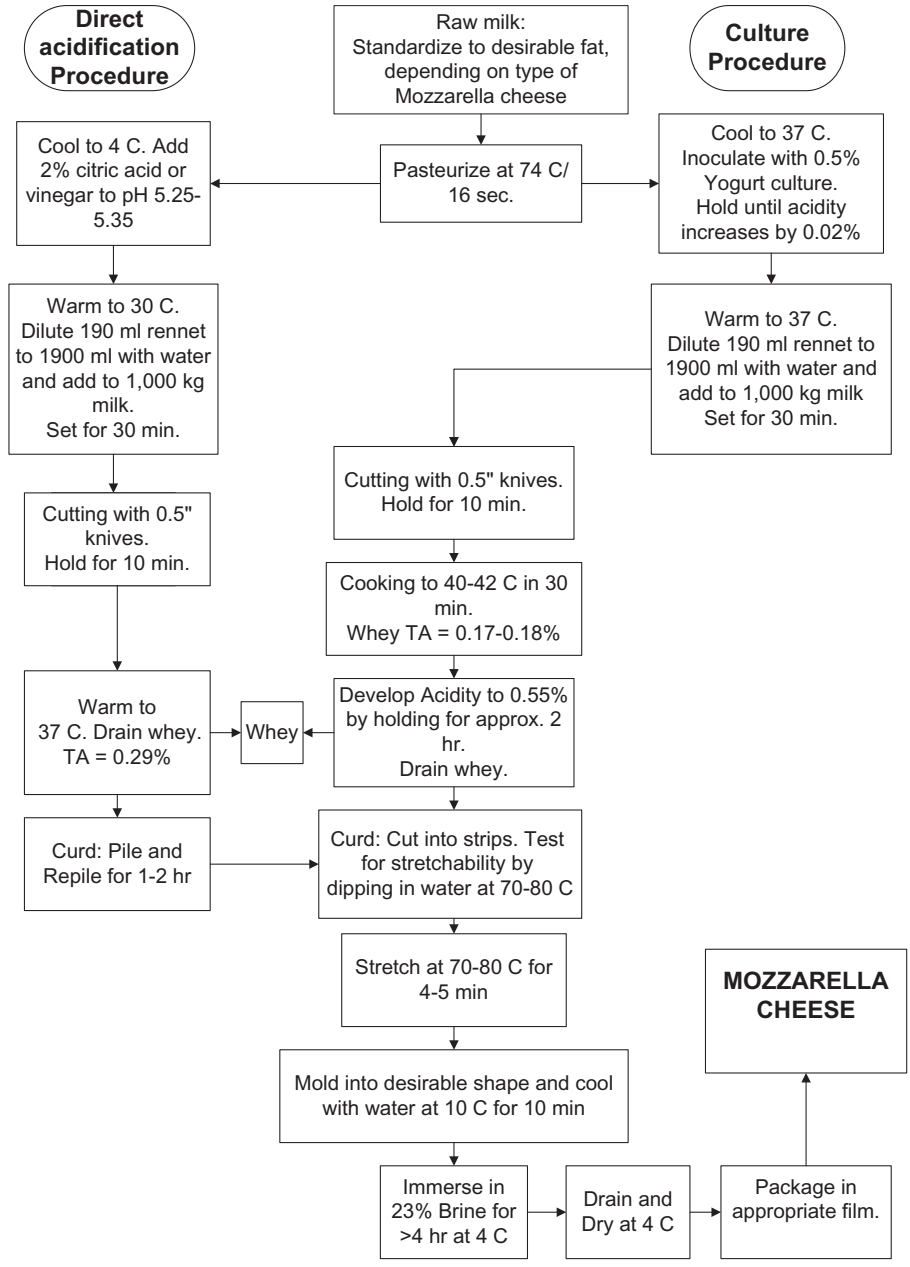

Figure 11.3. Flow diagram to illustrate the manufacture of mozzarella cheese by direct acidification and traditional culture processes.

about 10 minutes before agitation is started. The curd-whey mixture is cooked by raising the temperature of the vat's contents to 40°C to 42°C (104°F to 108°F) in 30 minutes. The rate of temperature increase is slow in the beginning. After the cooking temperature is attained, the curd-whey mixture is stirred for 10 minutes and agitation is stopped. Approximately 33% to 50% whey is drained out of the vat. Stirring is resumed for five to 10 minutes. The curd is allowed to settle for 10 minutes, followed by agitation for another 10 minutes. The curd is pushed away from whey exit end and whey is drained out. The curd is trenched and piled along the vat length. The total stir-out period is 30 to 45 minutes.

The individual blocks of cheese curd should be trimmed with a long knife. When the whey TA reaches 0.19%, the slabs are cut into 6- to 8-inch-long blocks and turned every 10 to 15 minutes. The total time for turning is about 90 minutes. Following packing for 30 to 45 minutes, the blocks of curd are piled two high. After 30 minutes, they are piled three high until the body of the curd is transformed from a soft, springy, granular character to a plastic, smooth, and fibrous state. At this point, the blocks tend to flow a little and acquire a smooth surface, with a few or no mechanical openings. When the TA reaches 0.8% to 0.9%, the curd is ready for milling.

It is milled through a mechanical cutter to 2 to 3 inches in size. Alternatively, the curd is cut into thin ribbons by passing it through a rotary cutter. At this point, the curd should achieve stretching character, which is tested by immersing curd ribbons in water at 82°C (180°F), followed by kneading and pulling to determine its ability to form long coherent threads. This leads to the mixing with hot water and stretching step.

Mozzarella cheese manufacturers use stretching and molding machines in which the curd particles are treated with hot water at 82°C (180°F), followed by working the curd to achieve a smooth, elastic, and lump-free mass. The wash water is saved along with the whey to extract milk fat. Good stretching entails no marbling in the appearance of the curd. Improper or incomplete stretching leads to marbling of the curd and is associated with water at low temperature and low curd acidity. The next step is molding the molten curd mass, which is now carried out in the stretching-molding machine. If done by hand, the curd is immersed in equal volume of hot water (70°C to 80°C; 158°F to 176°F), kneaded like dough, fused, and stretched like taffy. The internal temperature of the cheese should reach at least 50°C (122°F)—but it should not exceed 60°C (140°F)—and the pH should be in the range of 5.0 to 5.3.

The curd is then worked more and molded into the desired shapes and sizes. The molded cheese form is then cooled by immersion in cold water at 10°C (50°F) or salt solution. The cooling process results in firming of the cheese block and may take as long as an hour, depending on the size of the cheese loaf.

It is then ready for immersion in 22% salt solution at 5°C to 6°C (41°F to 43°F) in a brine tank. The time allowed for brining is one to three days, depending on the size and shape of the curd mass. The finished cheese should test 1.5% to 1.6% salt. After brining, the cheese blocks are dried in a cold room until drip-dry and packaged in shrinkable film. Mozzarella cheese does not require any curing and may be shredded and used soon after it is manufactured. However, storage in a cold room for 10 to 12 days improves the melting behavior of mozzarella/pizza cheese.

Mozzarella cheese is a popular cheese for pizza and Italian foods. The functional properties of mozzarella cheese are important for the application. Pizza made with mozzarella displays desirable stretch, melt, browning, oiling-off, and surface blisters. The chemical composition of the cheese determines the functionality of the cheese. The composition is controlled by strict control of cheese pH, calcium content, fat content, moisture level,

salt content, lactose/galactose content, and proteolytic activity during storage (Chandan, 1997).

- Stretchability depends on the calcium-phosphate level and proteolysis in cheese. The calcium content is controlled by acidity developed at the time of whey draining and at the hot-water stretching stage. The conversion of dicalcium paracaseinate to monocalcium paracaseinate is engineered by controlling culture activity. It is important to limit further lactic acid production; otherwise, paracasein will be produced from monocalcium paracaseinate. Paracasein cannot hold milk fat, resulting in low-fat, poor-quality cheese. On the other hand, too much calcium (predominantly dicalcium paracaseinate) gives short stretch with a tough and grainy texture. Too much proteolysis in aged cheese also negatively influences stretchability.
- Meltability depends on the proteolytic activity of the culture, fat content, and moisture content of mozzarella cheese. Higher meltability is observed with higher fat and higher moisture content. Fresh mozzarella is relatively tough and elastic, but on ripening for about three weeks, it develops the desired texture and meltability character. Overmelting also is undesirable because melted cheese runs off the pizza.
- Browning results from the Maillard reaction between the free hydroxyl group of a carbohydrate (lactose or galactose) and a free amino group of an amino acid in milk protein. Proteolytic activity of the culture liberates more free amino groups. The growth of *Streptococcus thermophilus* involves hydrolysis of lactose to glucose and galactose. Glucose is metabolized by glycolysis to lactic acid. The process results in a net accumulation of galactose with the free hydroxyl group available for interaction with the free NH_2 group of hydrolyzed protein to yield brown pigment. Thus, browning of cheese on pizza is enhanced if too much galactose accumulates or too much lactose is retained or proteolysis is abnormal. Judicious selection of culture strains exhibiting non-browning character of pizza cheese is needed to minimize this defect.
- Oiling-off is undesirable because it imparts an oily surface to pizza. The fat freed by heat during baking may even run off the edges of pizza, giving an unsightly appearance. The fat content of the cheese and protein matrix strength are critical factors in this regard. Pizza cheese is generally made from part-skim milk to reduce excessive fat, which might otherwise oil-off. Aged cheese tends to oil-off more than fresh cheese due to the proteolysis factor. High acidification and enhanced salting generally reduce calcium content of pizza cheese, resulting in efficient emulsification. Accordingly, oiling-off is thereby reduced.
- Blistering (size, number, and color) is another functional attribute of mozzarella cheese during the baking process. Large blisters are undesirable. They are caused by excessive ripening of the cheese. A young cheese generally has a large number of small blisters.
- Shreddability is a function of fat and moisture content. Generally, low-moisture, part-skim mozzarella has satisfactory shredding properties. Chemical analysis for moisture, fat, salt, and pH should be performed on representative samples of each batch to ensure conformation to the product standards and specifications.

Finally, microbiological analyses should be recorded to ensure consumer safety. The coliform count should be less than 10 CFU/g. Cheese should show negative counts for *E. coli*, Staph. (Coagulase positive), Salmonella, and *Clostridium perfringes.* The mold and yeast count should be less than 100 CFU/g.

Provolone Cheese

Like mozzarella, provolone is also a pasta filata, or stretched-curd-type cheese. It has less moisture than mozzarella and is additionally cured by suspending in ropes or plastic mesh at 85% humidity. It has a stringy texture. The minimum milk fat content is 45% by weight of the solids, and the maximum moisture content is 45%.

Provolone Cheese Process: Milk is standardized to a P/F ratio of 1.17, pasteurized, and tempered in a vat at 30°C (86°F). Green or blue color may be added to milk to neutralize the creamy color of cow's milk. Lipase preparation is then added as per recommendations from the supplier, followed by 1% to 2% mesophilic and thermophilic culture. Rennet (190 ml/1,000 kg) is added after dilution with water (1 : 10). After setting for 30 minutes, the coagulum is cut with 0.62-cm (1/4-inch) knives. The mixture is gently stirred for 10 minutes and the curd is allowed to settle. At this stage, the procedure for cooking to 39°C (102.2°F) in 30 minutes is identical to that for mozzarella and cheddar cheeses.

After stirring the curd for 10 minutes, the curd is allowed to settle and 33% of the whey is drained. Agitation is resumed until the whey pH reaches 6.1 to 6.2. The agitation is then stopped and all the whey is removed. The curd is trenched along the vat and piled to form slabs. The slabs are cut and repiled until the pH reaches 5.4. The slabs are mechanically cut into ribbons or small pieces and stretched and molded as in mozzarella cheese.

Following the stretching step, provolone may be shaped as a cylinder, truncated cone, ball, sausage, or melon and floated in cold brine. After the curd forms or shapes are brined, the cheese is suspended in traditional smooth twine or rope or in plastic netting for drying, followed by curing at 7°C (45°F) in rooms with humidity around 85%. Provolone may be smoked in a cold room for two to four hours. Vacuum packing of cheese prevents mold growth on the cheese surface.

Provolone cheese should display a characteristic compact and thread-like texture. The flavor of provolone after a few months of ripening is mild and creamy, which changes to sharp and piquant upon further ripening.

Parmesan and Reggiano Cheese

The main types of grana or parmesan cheese in Italy are called Parmigiano Reggiano and grana Padano. In the United States, parmesan or Reggiano cheese is characterized by a granular texture. It may have a hard and brittle rind. It grates readily. According to the FDA regulations (CFR 2009a), this cheese contains not more than 32% of moisture, and its solids contain not less than 32% of milk fat. It is cured for not less than 10 months.

Parmesan Cheese Process: For the manufacture of parmesan cheese, milk is first standardized to 1.8% fat and pasteurized. An enzyme preparation aiding in the curing or development of flavor of parmesan cheese may be added during the procedure, in such quantity that the weight of the solids of such preparation is not more than 0.1% of the weight of the milk used. Milk may be bleached by the use of benzoyl peroxide or a mixture of benzoyl peroxide with potassium alum, calcium sulfate, and magnesium carbonate; but the weight of the benzoyl peroxide is not more than 0.002% of the weight of the milk bleached, and the weight of potassium alum, calcium sulfate, and magnesium carbonate, singly or combined, is not more than six times the weight of the benzoyl peroxide used. If milk is bleached in this manner, sufficient vitamin A is added to the curd to compensate for the vitamin A or its precursors that are destroyed in the bleaching process. Artificial coloring is not allowed. Milk is set at 32°C to 35°C (90°F to 95°F). Natural cheese color may be added.

The starter, consisting of themophilic organisms *Streptococcus thermophilus* and

Lactobacillus delbrueckii subsp. *bulgaricus,* is added at the 1% level. Sometimes mesophilic culture is also used. After a ripening period of 10 minutes, rennet is added at the rate of 190 ml/1,000 kg of milk. Prior to its addition, the rennet is diluted 10 times with water. The milk coagulates in about 20 minutes. The relatively soft coagulated mass is cut two times with 0.62-cm (1/4-inch) knives and agitated to create curd pieces no larger than wheat kernels. The TA of whey should be 0.11% to 0.115% and its fat content should be 0.4% to 0.6%.

To prevent excessive fat loss, the curd particles are left undisturbed for 10 minutes. The curd is then heated, with slow agitation at the beginning. The temperature of the vat should reach 42.2°C (108°F) in 15 minutes. This temperature should be maintained for 15 minutes with vigorous agitation. Stirring is continued until the temperature reaches between 51.7°C and 54.4°C (125°F to 130°F). The TA of whey at this stage should be 0.125% to 0.13%. The curd is allowed to settle, pushed to the end to form a heap, and about half of the whey is drained. The acidity should continue to build to 0.17% to 0.19%. Then, all of the whey is removed.

The curd is cut and transferred to Daisy hoops. The cylindrical hoops are turned and pressed for 10 minutes, followed by turning and pressing again for 45 minutes. Cheese blocks are then removed from the hoops and placed on the draining surface. They are turned every hour, three times, and allowed to cool to 21°C to 25°C (70°F to 76°F) overnight. The pressed curd is salted for about two weeks in brine or dry-salted. The cheese is dried and cured in a cool, ventilated room at 10°C (50°F) for at least 10 months. In older plants, the rind of the cheese may be coated with vegetable oil or colored.

Romano Cheese

Romano cheese is prepared from cow's milk, sheep's milk, or goat's milk, or mixtures of two or all of these. It grates readily, and has a granular texture and may have a hard and brittle rind. It contains not more than 34% of moisture, and its solids contain not less than 38% of milk fat.

Romano Cheese Process: Milk is standardized to a P/F ratio of 1.5, pasteurized, and tempered to 32°C (89.6°F) in the cheese vat. The starter, consisting of equal proportions of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus,* is added at the 1.5% level. After 15 minutes, lipase preparation is added according to the manufacturer's instructions, followed by addition of rennet at the rate of 190 ml rennet/1,000 kg of milk. As usual, rennet is diluted 10 times. After the curd is set, it is continuously cut with 0.62-cm (1/4-inch) knives until the curd is of the size of rice grains. Cooking is started to raise the temperature to 46°C (114.8°F) in a span of 50 minutes. When the pH drops to 6.1 to 6.2, the curd is pushed away from the exit gate and leveled below the whey surface. Next, the whey is drained and the curd is filled into hoops. After 20 minutes, the hoops are stacked double for another 20 minutes. They are reversed and kept for 20 minutes, followed by pressing for 60 minutes. The hoops are removed from the press and stored overnight at room temperature. The cheese blocks are retrieved from the hoops and are placed in brine for two to three days, followed by drying at 10°C (50°F) for two days. The cheese is packaged in barrier-properties film and ripened at 10°C to 15°C (50°F to 60°F) for five to six months.

Soft-Ripened Cheese Group

Table 11.6 shows the chemical composition of soft-ripened cheeses.

Feta Cheese

Feta cheese is traditionally made from sheep milk and is naturally white in appearance.

Table 11.6. Approximate chemical composition of some soft- ripened cheeses.

Cheese	pH	% Moisture	% Fat	% Fat in dry matter	% Protein	% Salt	% Lactose
Feta	5.6	55.7	20.3	47.4	13.4	2.2	4.1
Camembert	5.7	52.5	23.0	50.4	18.5	2.5	0.4
Brie	5.8	48.4	27.7	53.7	20.7	2.3	0.4

Adapted from Fox et al. (2000a), Nath (1993)

Goat and buffalo milk also produce white cheese. However, cow's milk should be treated with approximately 0.04% titanium dioxide to simulate the white color; it is added to milk before rennet treatment. Milk is standardized to a P/F ratio of 0.9 by adding more fat to cow's milk, pasteurized under standard conditions, and set at 30°C (86°F). Starter containing *Lactococcus lactis* ssp. *lactis/cremoris* is added at the 3% level along with lipase preparation at the level of 3 g/1,000 kg of milk. The TA is allowed to increase by 0.05% and then rennet is mixed in at 120 ml/1,000 g milk. The milk sets in 45 to 60 minutes. The curd is cut with 1.27-cm (1/2-inch) knives and stirred gently for 20 minutes. The curd is dipped into rectangular molds, drained for two hours, and stored overnight in a room maintained at 18°C (64°F) and 85% relative humidity. The curd should achieve pH of 4.7 in about 24 hours. It is then cut into 10-cm cubes and salted at 50 g salt/kg of cheese. It may be brine salted in drums containing 8% brine and ripened at 8°C to 10°C (46°F to 50°F) for about 30 days. Feta cheese is generally stored at 2°C (36°F). It may be sold in blocks, cubes, or crumbled form.

Feta cheese can be made from ultrafiltered (UF) milk (40% solids). This process essentially avoids the whey drainage step. The UF concentrate is inoculated with 3% culture and 250 ml of rennet/1,000 kg of concentrate. The mixture is dispensed into 1-liter molds and ripened to pH of 4.8. Salt is added at the rate of 3% (cheese basis). The cheese is ready after ripening at 18°C (64°F) for one week.

Camembert/Brie Cheese

Camembert/Brie cheeses are soft-ripened cheeses in which white surface mold *Penicillium camemberti/candidum* gives them their characteristic appearance and flavor. Camembert cheese has about 52% moisture and the fat content in dry matter is 50%, whereas Brie has about 48% moisture and the fat content in dry matter is about 54%. The process for making both cheeses is similar, except the fat content of starting milk is higher for Brie.

Camembert Cheese Process: Milk is standardized to a P/F ratio of 0.86, pasteurized, and tempered to 32°C (90°F) in a cheese vat. It is then inoculated with 3% starter consisting of mesophilic culture of *Lactococcus lactis/cremoris*. The spores of the mold also may be inoculated. After one hour the TA should increase by 0.05%. At this stage, rennet at the rate of 250 ml/1,000 kg milk is added after dilution with 10 volumes of water. The mix is agitated for 5 minutes to mix the rennet and milk is allowed to set without agitation.

When the pH drops to 6.2 to 6.3 in about an hour, the coagulum is cut using 1.27-cm (1/2-inch) knives and the curd is allowed to settle for an hour. The whey is drained to reach the curd level. Dipping of the curd-whey mixture in cylindrical molds is begun. The molds are filled to within 1 to 2 cm from the top and the whey draining continues. The soft curd compacts. The molds are turned upside down four to six times within four to six hours and occasionally after that. It takes 12 to 18 hours for the pH of the whey to reach

4.6 to 4.9. The cheese disks are taken out of the hoops and dry salted by rubbing salt on all sides.

The salted cheese is stored in a curing room with 85% relative humidity at 12°C to 14°C (54°F to 57°F) for 24 hours. At this point the white mold culture suspended in water is sprayed on all sides of the cheese. After six to 12 days the cheese surfaces exhibit luxurious growth of the white mold. The pH should rise to above 7.0. The cheese is packaged in foil or waxed paper and stored at 4°C (39°F). It is ready for consumption when it becomes soft. The cheese surface should have a regular disc-like shape with a thin rind and snow-white surface. The body should be smooth with light-creamy color and display no runny character. The flavor should be pleasant, mild, and characteristic of the cheese. It must not exhibit soapy, bitter, metallic, or pungent notes. The over-ripened cheese turns slightly brown in color, becomes runny, and displays an ammonia odor.

Dutch Cheese Group

The chemical composition of Dutch- and Swiss-type cheeses is given in Table 11.7.

Edam and Gouda Cheeses

Edam and Gouda cheeses are typical Dutch-type cheeses. Edam cheese contains a minimum milk fat content of 40% by weight of the solids (FDM) and the maximum moisture content is 45%. Gouda has slightly more fat (46% FDM) than edam (40% FDM). The maximum moisture content requirement is 45% in both. Their texture shows small holes, or eyes, ascribed to the use of a CO_2 gas generating culture.

Gouda Cheese Process: Milk is standardized to a P/F ratio of 1.07, pasteurized, and tempered to 32°C (90°F). Annatto coloring (1 to 2 ml/1,000 kg of milk) is generally used in the winter to standardize the cheese color. Next, the starter is incorporated at the 0.75% level. The starter contains *Lactococcus lactis* subsp. *lactis* and *Leuconostoc cremoris. Lactococcus lactis subsp. diacetylactis* may be used sometimes. When the TA rises by 0.05% to 0.10%, rennet (190 ml/1,000 kg of milk) is diluted with 10 volumes of water and stirred in. When the coagulum is ready, it is cut into 0.5- to 1-cm cubes in 10 to 15 minutes. The curd is agitated for 20 to 30 minutes until the pH drops to 6.4 to 6.5.

At this point, 33% of the whey is drained off, followed by addition of sufficient water at 60°C (140°F) to warm the curd-whey mixture to 36°C to 38°C (96.8°F to 100.4°F). Water should be added gradually with agitation in 15 to 20 minutes. Agitation is continued for an additional 15 minutes, after which the curd is allowed to settle. The curd is covered with steel plates for pressing on the draining table. After the curd is pressed, the whey is removed. Liners are placed in appropriate size hoops and the curd is transferred into the hoops. The hoops are then pressed at 14 psi, turned, and then pressed at 14 to 28 psi. The hoops are turned occasionally. The pH after pressing should be 5.3 to 5.5.

The pressed cheese is taken out of the hoops and placed in 20% brine for salting.

Table 11.7. Approximate chemical composition of some Dutch and Swiss cheeses.

Cheese	pH	% Moisture	% Fat	% Fat in dry matter	% Protein	% Salt	% Lactose
Gouda	5.8	41.5	27.4	46.9	25.0	2.0	2.2
Edam	5.7	43.0	24.0	42.1	26.1	2.0	2.1
Swiss	5.6	37.2	27.4	43.7	28.4	1.2	3.4
Gruyere	5.7	33.5	30.0	50.4	30.0	1.1	2.9

Adapted from Fox et al. (2000a), Nath (1993)

Depending on the size, it is kept in brine for up to seven days for 20-kg blocks and for 20 hours for the 500-g size. Baby gouda is generally an 8-oz disc that requires only two hours of brine immersion. The cheese is packaged in plastic film and/or dipped in paraffin wax and aged for four to six weeks at 15°C (59°F). The pH should increase after ripening to 5.4 to 5.6. Gouda can be stored for six to 12 months at 10°C (50°F).

Swiss Cheese Group

Swiss/Emmentaler Cheese

Swiss or Emmentaler cheese has well-developed holes, or eyes, throughout. The minimum milk fat content is 43% by weight of the solids and the maximum moisture content is 41% by weight. Swiss cheese is ripened for at least 60 days. Benzoyl peroxide or a mixture of benzoyl peroxide with potassium alum, calcium sulfate, and magnesium carbonate may be used to bleach the dairy ingredients. The weight of the benzoyl peroxide must not exceed 0.002% of the weight of the milk being bleached, and the weight of the potassium alum, calcium sulfate, and magnesium carbonate, singly or combined, must not exceed six times the weight of benzoyl peroxide used. If milk is bleached in this manner, vitamin A is added to the curd in such quantity to compensate for the vitamin A or its precursors that are destroyed in the bleaching process, and artificial coloring is not used. Another bleaching agent, hydrogen peroxide, may be used, followed by a sufficient quantity of catalase preparation to eliminate the hydrogen peroxide. The weight of the hydrogen peroxide shall not exceed 0.05% of the weight of the milk and the weight of the catalase shall not exceed 20 ppm of the weight of the milk treated.

"Swiss cheese for manufacturing" conforms to the definition and standard of identity prescribed for Swiss cheese except that the eyes have not developed throughout the entire cheese. This product is used in process cheese manufacture.

Swiss Cheese Process: Milk is standardized to a P/F ratio of 1.1 by removing cream or adding liquid skim milk. The milk is tempered to 37°C (99°F) and inoculated with starter containing 0.1% *Streptococcus thermophilus*, 0.1% *Lactobacillis helveticus,* and 0.05% *Propionibacterium shermanii*. After 10 to 15 minutes, 190 ml rennet/1,000 kg of milk is added in diluted form. The rennet is diluted 10 times with water. After setting to a firm coagulum, a vigorous cutting operation is continued using 0.62-cm (1/4-inch) knives until the curd assumes the size of wheat grains. Clumping of the curd should be avoided. The curd is stirred for 30 to 60 minutes until it assumes a firm and resilient character.

Next, the curd is cooked by bringing the temperature to 52°C (126°F) in 30 minutes. The cooking temperature is raised slowly (1°C to 1.5°C/5 minutes; 1.8°F to 2.7°F/5 minutes) with agitation to avoid matting. The acidity should build and the pH should drop to 6.3 to 6.4. The stirring is suspended and the curd is allowed to settle. Sufficient whey is pumped out but the curd should not be exposed to air. The submerged curd is covered with cloth and press-plates. Weights are placed on the plates' surface. The whey is drained after 15 to 30 minutes. Pressing of the curd is continued for 12 to 18 hours. The cheese pH should drop to 5.2 to 5.4.

The plates and weights are removed and the curd is cut into blocks which are subsequently salted in saturated brine for 48 to 72 hours. The blocks are dried and packaged in special film to permit expansion due to gas formation during ripening. The blocks are ripened by storing them at 10°C (50°F) for five to 10 days, followed by storage at 23°C (73°F) for two to four weeks to permit eye formation and flavor development. Subsequently, the blocks are stored at temperatures below 5°C (41°F) to contain eye

Table 11.8. Approximate chemical composition of mold ripened (blue-veined) cheeses.

Cheese	pH	% Moisture	% Fat	% Fat in dry matter	% Protein	% Salt	% Lactose
Blue/Bleu	6.5	42.4	28.7	49.9	21.4	4.5	2.3
Roquefort	6.4	39.9	30.9	50.5	21.5	3.5	2.0
Gorgonzola	6.3	36.0	32.0	50.0	26.0	4.0	2.2
Nuworld	6.3	43.0	28.8	50.5	21.3	3.9	2.2
Stilton	5.2	38.3	33.0	53.5	24.8	3.5	2.2

Adapted from Fox et al. (2000a), Nath (1993)

formation. The cheese is now ready for retail cutting and packaging. It has the characteristic sweet nutty flavor.

Gruyere Cheese

Gruyere cheese contains small eyes. It has a mild flavor due in part to the growth of surface-curing agents. The minimum milk fat content is 45% by weight of the solids and the maximum moisture content is 39% by weight. The dairy ingredients used may be pasteurized. The cheese is ripened for at least 90 days.

Gruyere Cheese Process: Cheese milk is warmed and subjected to the action of lactic-acid-producing and propionic-acid-producing bacterial cultures. Rennet is added for coagulating milk. The coagulum is cut into particles similar in size to wheat kernels. For about 30 minutes the particles are alternately stirred and allowed to settle. The temperature is raised to about 52.2°C (126°F) and stirring continues until the curd becomes firm. The curd is transferred to hoops or forms, and pressed until the desired shape and firmness are obtained. The cheese is surface-salted while held at a temperature of 8.9°C to 12.2°C (48°F to 54°F) for a few days. It is soaked for one day in a saturated salt solution. It is then held for three weeks in a salting cellar and wiped every two days with brine cloth to ensure growth of biological curing agents on the rind. It is then removed to a heating room and held at progressively higher temperatures, finally reaching 18.3°C (65°F) with a relative humidity of 85% to 90%, for several weeks, during which time small holes form. The cheese is then stored at a lower temperature for further curing.

Blue Cheese Group

The chemical composition of mold-ripened cheeses is shown in Table 11.8.

Blue Cheese

Blue cheese is characterized by veins of bluish-green mold, *Penicillium roqueforti*, throughout the cheese. The minimum milk fat content is 50% by weight of the solids and the maximum moisture content is 46%. Blue cheese is ripened for at least 60 days. It is made from cow's milk.

Among the other optional ingredients for blue cheese is blue or green color in an amount to neutralize the natural yellow color of the curd. Benzoyl peroxide or a mixture of benzoyl peroxide with potassium alum, calcium sulfate, and magnesium carbonate may be used to bleach the dairy ingredients. The weight of the benzoyl peroxide is not more than 0.002% of the weight of the milk being bleached, and the weight of the potassium alum, calcium sulfate, and magnesium carbonate, singly or combined, is not more than six times the weight of the benzoyl peroxide used. If milk is bleached in this manner, vitamin A is added to the curd in such a quantity to compensate for the vitamin A or its precursors that are destroyed in the bleaching process, and artificial coloring is not used.

Blue Cheese Process: The milk is standardized to a P/F ratio of 0.87, and pasteurized. Milk may be homogenized to promote fat hydrolysis. Alternatively, 30 g of lipase preparation is added per 1,000 kg of milk. Milk may be whitened by adding 0.03% to 0.04% titanium dioxide to simulate the white color of ewe's milk. Starter containing *Lactococcus lactis* subsp. *lactis/cremoris* is added at the 3% level and the milk is set at 32°C (90°F). After an hour, the pH should drop by 0.05 to 0.1 pH unit (increase in TA of 0.05%). Then rennet is added (200 ml/1,000 kg of milk, diluted 20 times with water) and allowed to coagulate.

After setting for an hour, the curd is cut with 1.27-cm (1/2-inch) knives and allowed to settle to the bottom of the vat. After 10 minutes, slow stirring is started. When the TA increases by 0.01% as compared to the cutting TA, the whey is drained enough to expose the curd. The curd is broken and all of the whey is drained off. The curd is ditched along the sides of the vat and turned over after 10 minutes. Salt is added to the curd at the 1% level, mixed, and inoculated with powder containing spores of blue mold, *Penicillium roqueforti*. The curd is transferred to cylindrical hoops placed on the draining table. The molds are turned every five to 10 minutes in the initial stages and later at 30-minute intervals. They are incubated at room temperature for 16 to 20 hours, until the pH drops to 4.5 to 4.7.

At this point, the cheese forms are removed from the hoops and salt (50 g salt/kg cheese) is rubbed over the cheese surface. The salted cheese is stored at 12°C to 13°C (54° to 55°F) for 24 hours. It is turned and brushed for smear development. The cheese is then mechanically perforated (approximately 60 holes) on the top and bottom with a 3-mm skewer to facilitate oxygen availability for mold growth. Cheese blocks are ripened at 95% relative humidity for six to eight weeks at 12°C to 14°C (54°F to 57°F). Mold growth should be visible at this point. The surface of the cheese may be scraped to remove surface growth of undesirable organisms. After ripening, the pH should increase to 6.0 to 6.2. Blue cheese is vacuum packed and ready for market. In some cases, vegetable fats may be used to coat the rind. Antimycotics may be applied to the surface of bulk cheese during ripening and to cheese cuts prior to packaging in films.

Roquefort Cheese

Roquefort cheese also can be called sheep's milk blue-mold cheese or blue-mold cheese from sheep's milk. It is characterized by the presence of bluish-green mold, *Penicillium roqueforti,* throughout the cheese. The minimum milk fat content is 50% by weight of the solids and the maximum moisture content is 45%. Roquefort cheese is ripened for at least 60 days. The process resembles the blue cheese process above.

Gorgonzola Cheese

Gorgonzola cheese is characterized by the presence of bluish-green mold, *Penicillium roqueforti*, throughout the cheese. The minimum milk fat content is 50% by weight of the solids and the maximum moisture content is 42%. Gorgonzola cheese is made from milk of either cows or goats or mixtures of the two milks. In contrast, blue cheese is made exclusively from pasteurized cow's milk. Gorgonzola is ripened for at least 90 days, whereas blue cheese can be sold after ripening for 60 days.

Gorgonzola Cheese Process: The process resembles the manufacture of blue cheese, given above. Milk (goat, cow, or a mixture thereof) is pasteurized. Lipases may be used for flavor development and blue or green color may be added to neutralize the creamy yellow color of cow's milk. In addition, bleaching agents (benzoyl peroxide) also may be used. The starter, consisting of mesophilic culture, is added to the milk, followed by rennet addition to develop coagulum. The

Table 11.9. Approximate chemical composition of some semi-soft, ripened cheeses.

Cheese	pH	% Moisture	% Fat	% Fat in dry matter	% Protein	% Salt	% Lactose
Brick	6.4	41.1	29.7	50.4	23.3	1.9	1.8
Munster	5.7	41.8	30.0	51.6	23.4	1.9	1.1
Monterrey Jack	5.7	42.0	29.6	51.0	23.5	2.0	2.8

Adapted from Olson (1969), Fox et al. (2000a), Nath (1993)

coagulum is then cut into small curd particles and dipped into perforated forms. The mold culture of *Penicillium roqueforti* and its spores is mixed into the curd-whey mixture. The whey drains out of the forms to form a compact form which is then removed from the hoops and dry salted. The ripening takes place in a room held at 10°C (50°F) with 90% to 95% humidity until the characteristic blue-green mold develops.

Nuworld Cheese

Nuworld cheese is characterized by the presence of creamy-white mold, a white mutant of *Penicillium roqueforti*, throughout the cheese. The minimum milk fat content is 50% by weight of the solids and the maximum moisture content is 46%. Nuworld cheese is made from pasteurized dairy ingredients and is at least 60 days old. The manufacturing process is similar to that of blue cheese, given above. The curd and whey mixture is placed into forms permitting further drainage. While being placed in the forms, spores of a white mutant of the mold *Penicillium roqueforti* are added. The forms are turned several times during drainage. When sufficiently drained, the shaped curd is removed from the forms and salted with dry salt or brine. Perforations are then made in the shaped curd and it is held at a temperature of approximately 10°C (50°F) at 90% to 95% relative humidity, until the characteristic mold growth has developed. During storage, the surface of the cheese may be scraped to remove surface growth of undesirable microorganisms. Nuworld cheese has characteristic sharp blue cheese flavor without the color of blue cheese.

Semi-soft Ripened Cheese Group

The composition of semi-soft ripened cheeses is given in Table 11.9.

Brick Cheese

Brick cheese is a semi-soft variety that contains at least 50% fat in dry matter and not more than 44% moisture. Normally, the salt content is 1.5%. The cheese is cured at a temperature of not less than 1.7°C (35°F) for at least 60 days. Addition of cheese color is optional. Cheese milk is brought to a temperature of 31.1°C (88°F) and subjected to the action of a lactic acid-producing bacterial culture. One or more of the clotting enzymes is added to set the dairy ingredients to a semi-solid mass.

The mass is cut into cubes with 0.95-cm (3/8-inch) knives, stirred, and heated so that the temperature rises slowly to 35.6°C (96°F). The stirring is continued until the curd is sufficiently firm. Part of the whey is then removed, and the mixture is diluted with water or salt brine to control the acidity. The curd is transferred to forms and drained. During drainage it is pressed and turned. After drainage, the curd is salted and the smear culture (*Brevibacterium linens*) is applied to the surface. The cheese is then cured to develop the characteristics of brick cheese.

Brick cheese for manufacturing conforms to the definition and standard of identity for brick cheese except that the dairy ingredients are not pasteurized and curing is not required.

Brick Cheese Process: Milk is pasteurized, tempered to 32°C (90°F) in a cheese vat, and inoculated with 0.25% *mesophilic*

Lactococcus culture and 0.25% *Streptococcus thermophilus* (Nath, 1993). When the TA reaches 0.18%, rennet (170 ml/1,000 kg of milk) diluted with 10 volumes of water to set the coagulum in 30 minutes. The coagulum is cut with 0.62-inch (1/4-inch) knives. Next, the curd-whey mixture is heated gradually to achieve a vat temperature of 36°C (97°F). The whey is drained to leave an inch over the curd surface and water at 36°C (97°F) is added to replenish the drained whey volume. After five minutes, the whey-water mixture is drained to the level of the curd.

The curd-whey-water mixture is pumped to rectangular hoops, which are covered and turned after one hour; 5-lb weight is then applied. The hoops are turned three more times at one-hour intervals. The room temperature should be 21°C to 24°C (70°F to 75°F). The weight is removed after the fourth turn. The loaves of cheese are removed from the hoops and floated in a brine tank at 10°C (50°F) for 24 hours. Dry salt is sprinkled on the surface of loaves, turning them after 16 hours. The pH of cheese after one day should be 5.2 to 5.3. The salted loaves are ripened at 15.6°C (60°F) with 90% humidity.

Cheese cloth containing *B. linens* smear is then applied to the surfaces of cheese loaves. Each day the loaves are turned and hand rubbed with 5% salt water. The cheese loaves are ripened for five to 10 days, depending on the desired intensity of flavor. The smear is washed off and the loaves are dried in the ripening room. They are packaged in plastic film and sealed. Further ripening is carried out at 4.4°C (40°F) for four to eight weeks. If desired, the pungency of cheese flavor can be enhanced by skipping the wash treatment.

Muenster Cheese

In Europe, Muenster (or Munster) is a surface-ripened cheese. However, in the United States, Muenster cheese involves no smear ripening. It is a semi-soft cheese with a minimum of 50% fat in dry matter and the moisture content is 46% or less by weight. Muenster cheese for manufacturing conforms to the definition and standard of identity for Muenster cheese except that the dairy ingredients are not required to be pasteurized. It can be used for process cheese formulation.

Muenster Cheese Process: The process resembles that of brick cheese manufacture, except that no smear treatment is applied and adjustments are made to raise the moisture content. Milk is pasteurized and tempered to 32.2°C (90°F) in a cheese vat. Starter consisting of *Streptoccus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* is added at the 0.5% to 1% level. Rennet (at the level of 2 to 3 oz/1,000 lb of milk) is diluted and added soon after. After 30 minutes of setting, the coagulum is cut with 0.95-cm (3/8-inch) wire knives and heating is started after 5 minutes. The rate of heating is initially slow to avoid case-hardening of the curd. The cooking is complete when the temperature reaches 40°C to 41°C (103°F to 106°F). The pH of the curd-whey mixture should be 6.57 (TA of whey 0.10% to 0.105%). The whey is drained until the curd level is visible.

The vat contents are pumped to hoops on the draining table, and the hoops are turned after 20 minutes. The second and third turning of hoops is done after an hour each. After the third turn, the pH of the whey should be 5.75 (TA of 0.35% to 0.45%). When the pH reaches 5.50 (TA of 0.55% to 0.65%), the cheese wheels or bricks are removed from the molds and immersed in a cold 22% brine for 24 hours. They may be coated with annatto to give them a yellow appearance. They are wrapped in film and ripened for three to six weeks.

Unripened Fresh Cheeses

In most cheeses, the curd formation is carried out with or without fermentation by the application of coagulating enzymes isolated from various biological sources. However, certain cheese varieties do not use enzymes. Milk coagulation is characterized by an acid

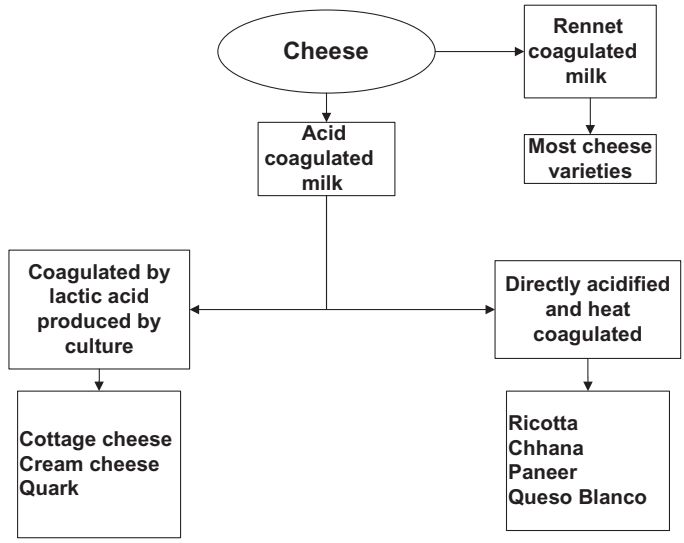

Figure 11.4. Acid-coagulated cheeses and their classification.

generated *in situ* by an added culture or by direct addition to hot milk. Such cheeses do not involve ripening and are consumed as fresh cheeses.

Figure 11.4 shows the classification of acid-coagulated cheeses. There are two types, according to the coagulating procedure used. In the first type, coagulation of milk at ambient temperatures is accomplished by lactic acid produced as a result of fermentation with lactic acid bacteria. Cottage cheese, quark and cream cheese are examples of first type of acid coagulated cheeses. The second type is produced by direct acid addition and heat. This type includes ricotta, chhana, paneer, and queso blanco.

Cheeses Obtained by Acidification with Culture

Table 11.10 displays the chemical composition of cultured, acid-coagulated cheeses.

Cottage Cheese: Cottage cheese is the soft uncured cheese prepared by mixing cottage cheese dry curd with a creaming mixture. It is a Grade A product (USDHHS FDA, 2003). The milk fat content of cottage cheese is not less than 4% by weight of the finished food and the moisture content is not more than 80%. Cottage cheese with lower fat content (1%) is also available. In addition, cottage cheese dry curd containing less than 0.5% milk fat and no more than 80% of moisture is marketed as cottage cheese dry curd or dry curd cottage cheese. The dairy ingredients used for manufacturing this cheese are skim milk, concentrated skim milk, and nonfat dry milk. If concentrated skim milk or nonfat dry milk is used, water may be added in a quantity not in excess of that removed when the skim milk was concentrated or dried.

This cheese is made from pasteurized skim milk, concentrated nonfat milk or reconstituted nonfat dry milk. Calcium chloride may be added to the milk at a level not to exceed 0.02% (calculated as anhydrous calcium chloride) of the weight of the milk. A small amount of rennet may be added. Coagulation of skim milk is accomplished by fermentation with *Lactococcus lactis* subsp. *lactis* or *Lactococcus lactis* subsp. *cremoris.*

Table 11.10. Approximate chemical composition of various cultured, acid–coagulated cheeses.

Cheese	pH	% Moisture	% Fat	% Protein	% Salt	% Lactose
Cottage, creamed, 4% fat	4.9	79.0	4.1	12.5	1.0	2.7
Cottage, 2% fat	4.9	79.8	2.0	14	1.0	2.8
Cottage, 1% fat	4.9	79.9	1.0	15	1.0	2.9
Cottage, fat free	4.7	79.9	<0.5	17.3	0	1.8
Bakers	4.5	74	0.2–0.6	19	<0.1	2.6
Cream, regular	4.6	53.7	34.9	7.5	0.7–1.2	2.7
Cream, low fat	4.6	64.1	13.5	13	0.7–1.2	2.5
Cream, fat free	4.6	76.0	<0.5	14	0.7–1.2	2.7
Neufchatel	4.6	62.2	23.4	10.0	0.75	2.9
Quark, creamed	4.4	73	12	10	0.1–0.7	2.6
Quark, low fat	4.5	82	0.5	13	0.1–0.7	2.7

Adapted from USDA (2002), Chandan (2003), Nath (1993), Lucey (2003)

The fermentation is continued to reach a pH of between 4.5 and 4.7. Coagulation to a firm curd is achieved while heating to a maximum of 48.9°C (120°F) without agitation. The coagulated mass may be cut and stirred, followed by whey draining. The curd is washed with water, stirred, and further drained. It may be pressed, chilled, worked, and seasoned with salt.

Alternatively, food-grade acids (hydrochloric, phosphoric, citric, lactic acids) or D-Glucono-δ-lactone with or without rennet may added in such amounts to reach a final pH in the range of 4.5 to 4.8. The mixture is held until it becomes coagulated. The coagulated mass may be cut, cooked, and drained and processed further as in the cultured process. However, the product must be labeled "directly set," "direct acid set," or "curd set by direct acidification."

To extend the shelf life of cottage cheese, sorbates are allowed. It may contain fruits, seafood, meats, and vegetables. It is marketed as small curd (less than 4-cm diameter) or large curd (greater than 8-cm diameter). Cottage cheese contains significantly lower fat content than most cheeses. It is a good source of protein and is a popular part of low-calorie diet. Cottage cheese is a major dairy product in the North America. In 2007, the United States production of cottage cheese curd, creamed cottage cheese, and low-fat cottage cheese totaled 460,630 million lbs; 360,748 million lbs; and 420,844 million lbs, respectively (USDA, 2008).

A good-quality cottage cheese should have a clean, creamy, cultured milk flavor; natural creamy color; and meaty, soft texture. It should have reasonably uniform curd size, and should not be pasty or too firm. It should have a uniform cream layer around the curd particles with a minimum of free cream. Depending on the plant schedule, short-set, medium-set, or long-set methods may be used. The long-set method calls for 1% starter and an incubation temperature of 22°C (72°F) for 12 to 16 hours. The short-set method uses fermentation with 5% to 7% starter at 32°C (90°F) for four to six hours. The intermediate-set method employs conditions between the two.

The manufacturing operation may consist of a simple cheese vat and equipment for whey removal, curd washing, draining, and creaming. The creamed curd is pumped to a filler for packaging. In general, large-scale operations employ automated systems to continuously carry out the various steps outlined below.

- Setting. Raw skim milk is pasteurized at 73°C for 16.5 seconds. It is desirable to avoid previously heated milk because a minimum of heat treatment is needed to avoid curd weakness and subsequent shattering of the curd. The pasteurized milk is

cooled to 32°C (89.6°F) and pumped into a cheese vat where the jacket temperature is adjusted to maintain the milk temperature at 32°C (89.6°F). Bulk culture containing *Lactococcus lactis* ssp. *lactis/cremoris* is added at the rate of 5% to 7% and mixed thoroughly. The TA/pH is measured at this point. The stirring is continued at intervals of 30 minutes for a period of 1.5 hours. The increase in acidity at this point should be 0.05% to 0.07%. If it is not, 1% more culture is added for each 0.01% increment below 0.05%. Next, rennet is added after diluting 1 part of rennet with 40 parts of water. Rennet is used at the rate of 1 ml for large curd and 0.3 to 0.5 ml per 454 kg (1,000 lbs) of skim milk. After mixing the rennet thoroughly, the vat is covered for about two hours for fermentation and the pH should drop to 4.6 to 4.7.

- Cutting. When the curd is ready, it is cut first along the length of the vat using appropriate horizontal knives to get the desired small- or large-curd product. Wire knives of 0.62 cm (1/4 inch) opening are used to for small-curd, and wire knives of 1.86 cm (3/4 inch) opening give a large-curd product. Next, the second cut is made lengthwise with the vertical knife. The third cut is made with the same vertical knife crosswise across the vat.
- Cooking. After cutting, the curd is allowed to heal for 10 minutes. The jacket water temperature is raised to 44°C to 50°C (111°F to 122°F) to initiate cooking of the curds. The temperature of the curd is raised slowly to 54°C (129°F) in 1.5 hours. The vat is hand-stirred every five to 10 minutes and a mechanical agitator is used after the temperature of the vat reaches 38°C to 41°C (100°F to 106°F). The agitation speed is increased slowly to avoid matting the curd. Small curd cooks faster than large curd. The agitation is continued until the curd becomes reasonably firm. The curd is adequately cooked when a handful of water-chilled curd springs apart when squeezed together with moderate pressure.
- Washing. After completion of cooking, the jacket water is drained. The whey is removed until the curd begins to surface. Wash water, acidified with phosphoric acid and glucono-δ-lactone to pH 4.6 and chlorinated to 10 to 20 ppm, is then added equal to the volume of the whey removed. The temperature of the curd should drop to 27°C to 30°C (81°F to 86°F). After agitation for 10 minutes, the water is drained. Washing is continued two more times with chilled water to drop the temperature of the curd to 13°C to 16°C (55°F to 61°F) and finally to 5°C (41°F) or lower. The curd is trenched and allowed to drain for 30 to 60 minutes before creaming.
- Creaming. The curd is blended with cream dressing in a blender or the vat itself. The dressing is formulated to contain 12.5% fat, 8.5% solids-not-fat (SNF), 2.7% salt, and 0.25% stabilizer. The stabilizer is especially designed for the dressing to display high viscosity and to avoid wheying off. The dressing is pasteurized at 75°C to 77°C (167°F to 171°F) with a holding period of 30 minutes and homogenized at 57°C (135°F) at a pressure of 136 atm (2,000 psi), single-stage followed by cooling to 4°C (39°F) or below. One part of the dressing is blended with two parts of the curd to yield a minimum of 4% fat in creamed cottage cheese. The dressing can be further treated to enhance the shelf life of cottage cheese to control the growth of spoilage psychrotrophic organisms, yeasts, and molds. Use of sorbic acid is allowed. The typical shelf life of cottage cheese packaged in moisture-barrier containers is three to four weeks at a storage temperature of 4°C (39°F).

Cottage cheese curd yield is typically around 15.5% kg/100 kg of skim milk of 9%

SNF. A fortified skim milk containing 12% SNF should give 21.6 kg of curd/100 kg of the starting material.

Quarg/Quark Cheese: This category of European fresh cheeses contains multiple levels of fat and moisture. Their manufacturing procedure resembles that of cottage cheese. Skim milk is pasteurized at 62°C (144°F) for 30 minutes, cooled to 32°C (90°F), and inoculated with 5% starter consisting of *Lactococcus lactis* ssp. *lactis/cremoris.* In four to six hours, the pH drops to 4.8 and a coagulum is formed. The curd is broken by stirring and cooked slowly to 50°C to 52°C (122°F to 126°F). The initial heating rate is only 0.5°C/5 minutes and the total cooking period is around 90 minutes until firm curd is obtained. The curd may be washed with chilled water (10°C; 50°F) to eliminate acid flavor. The curd is collected by centrifugation in a Quark separator and cooled by immersion in cold water for 15 minutes. The dried curd is blended with pasteurized, homogenized cream of 18% fat to achieve the fat target of 4% to 8% in the finished product. Bakers' cheese is also a variant of quark.

Cream and Neufchatel Cheese: Cream cheese is an important variety in North America. During 2007, cream and Neufchatel cheese production in the United States exceeded 772 million lbs (USDA, 2008). Cream cheese is mostly used as a spread and as an ingredient of cheesecakes. At present, cream cheese is marketed in flavors such as strawberry and other fruits. It is also flavored with vegetables, condiments, spices, and herbs. Low-fat versions are available as well. It is a popular spread on bagels and toasted bread. Cream cheese contains at least 33% fat and not more than 55% moisture. It is soft, unripened, lactic-acid-coagulated cheese, made by a process similar to that for cottage cheese. It has a mild acid and creamy flavor with buttery aroma.

Neufchatel cheese is also soft uncured cheese but contains lower fat and more moisture than cream cheese. Neufchatel cheese has a minimum fat content of 20%, but its fat content cannot exceed 33% by weight of the finished food. The maximum moisture content of Neufchatel cheese is 65%.

The dairy ingredients for cream and Neufchatel cheese are milk, nonfat milk, or cream used alone or in combination. Other ingredients approved by the FDA include rennet, salt, whey, concentrated whey, dried whey, or reconstituted whey prepared by addition of water. Stabilizers, in a total amount not to exceed 0.5% of the weight of the finished food, may be used. Stabilizers may contain dioctyl sodium sulfosuccinate in a maximum amount of 0.5% of the weight of the stabilizer used. The dairy and other ingredients are blended to standardize the mix for fat content.

Cream Cheese with Other Foods "Cream cheese with other foods" is another class of foods prepared by mixing, with or without the aid of heat, cream cheese with one or a mixture of two or more types of foods (except other cheeses) in an amount sufficient to differentiate the mixture from cream cheese. The maximum moisture content of the mixture is 60% by weight and the minimum milk fat is 33% by weight of the cream cheese and in no case less than 27% of the finished food. Foods permitted include properly prepared fresh, cooked, canned, or dried fruits or vegetables, meats, relishes, and pickles. Stabilizers are permitted at a level not to exceed 0.8%, with or without the addition of dioctyl sodium sulfosuccinate in a maximum amount of 0.5% of the weight of the stabilizer(s) used. Colors are also permitted.

Cream Cheese Process: The manufacturing procedures for cream and Neufchatel cheese are similar. Several processes, including ultrafiltered mix, are used to produce cream cheese. Typically, the process involves standardizing cream to 11% to 15% fat, followed by pasteurization at 68°C to 70°C

(154°F to 158°F)/30 minutes or equivalent. The mix is homogenized at 1,000 to 1,500 psi (6.9 to 10.3 MPa) at 63°C (145°F) and cooled to 32°C (90°F). Rennet is added at the rate of 1 ml/1,000 kg cream and blended. Using a starter consisting of *Lactococcus lactis* subspecies *lactis/cremoris* and *Leuconostoc crem*oris at the 5% to 6% level, the cream cheese mix is cultured at 30°C to 32°C (86°F to 90°F) for five hours. The long-set process employs 1% starter and incubation for 16 hours at 22°C (72°F). Addition of *Leuconostoc* sp. imparts a buttery aroma. The fermentation is continued until a pH of 4.4 to 4.5 is attained. Some processors use the thermophilic yogurt culture to acidify cream cheese mix to pH 4.4 to 4.6 at 43°C (109°F). However, the flavor lacks the buttery notes obtained with mesophilic culture. After the required pH is reached, the curd is agitated until it is lump free. Next, enough hot water at 76°C (169°F) is added to bring the temperature to 51°C (124°F). At this point, the curd should be smooth and creamy.

In the traditional process, the cultured cream is transferred to draining bags, which are dipped in hot water and allowed to hang and drain for two hours. However, most modern plants are equipped with centrifuges to separate whey. Cream cheese collects in the bowl. The curd is collected and salted to obtain 0.75% salt in the cheese. The yield of cream cheese should be 2.7 to 3.1 kg/kg of fat. The cheese is subsequently packaged by cold-pack procedure without any additives or heated and cooled to 2°C to 4°C (36°F to 39°F) for storage. The cold-pack cream cheese has more aroma and flavor, and no pasty/sticky body. However, it lacks the long shelf life obtained by the most commonly used hot-pack procedure. This procedure involves heat treatment of cream cheese in a kettle or scraped surface heat exchanger. Cream cheese is mechanically mixed with a stabilizer (0.35% locust bean gum) and 1% salt and brought to 70°C (158°F). The hot mixture is homogenized at 2,000 psi (13.79 MPa), followed by transfer to the packaging machine for hot packaging. The hot process imparts a shelf life of at least two months under refrigerated storage.

Cheeses Produced by Direct Acid Addition and Heat

In cheeses produced by direct acid addition and heat, the curd is formed by direct acidification of hot milk with food-grade acids. In general, the directly acidified cheeses are consumed in fresh or unripened form. These cheeses derived from milk coagulation with acid under sub-boiling temperatures are known by various names in different parts of the world. They are made from whole milk, low-fat milk, skim milk, cream, or whey or their mixtures. Milk of various species of animals is used in production of these cheeses. Depending upon its origin, curdling of hot milk may be carried out with vinegar, lactic acid, calcium lactate, lime/lemon juice, acid whey, or yogurt/fermented milk. In terms of commercial significance, ricotta, chhana, paneer, and queso blanco are discussed in this section.

Table 11.11 gives the proximate chemical composition of acid and heat-coagulated cheeses. The composition of cheddar cheese is given for comparison.

Ricotta Cheese: Ricotta, like cottage cheese, is a high-moisture cheese. Ricotta is not a pressed curd type of cheese. Its composition varies depending on whether it is made exclusively from whey or from a blend of whey and milk. Originally, ricotta was made from whey derived from mozzarella or provolone cheese production. Ricotta is now prepared from whole milk with or without the addition of whey. When made from a blend of 95% sweet whey and 5% milk, ricotta contains 68% to 73% moisture, 16% protein, 4% to 10% fat, and 4% lactose.

Ricotta has a bland to slightly cooked but pleasing flavor. Its texture is soft and creamy. It is consumed as such as a spread and may

Table 11.11. Approximate composition of directly acidified cheeses.

Cheese	pH	% Moisture	% Fat	% Protein	% Salt	% Lactose
Ricotta, whole milk	5.8	72	13	11	<0.5	2.9
Ricotta, part skim	5.8	74	8	12	<0.5	3.2
Ricottone	–	82	0.5	19	<0.5	3.3
Mascarpone	5.0	46	47	5	0	–
Chhana, cow's milk	5.7	53	25	17	0	2.2
Chhana, buffalo milk	5.4	52	27	14	0	2.3
Paneer, buffalo milk	5.8	51	26	17	0	2.3
Queso blanco	5.2	55	15–27	23	2.5	2.5
Cheddar	5.6	37	32	25	1.8	0

Adapted from Chandan (2007), Lucey (2003)

be used as a replacement for cream cheese or sour cream in dips. It is basically a non-melting cheese. Its major use is in Italian cuisine (e.g., lasagna and ravioli) and confectionery.

Ricotta Cheese Process: Most of the ricotta production is confined to a batch process. Traditionally, open kettles are used. Heating may be direct, or steam jackets may provide heat transfer. Sweet whey from Italian cheese manufacture is suitable as long as its pH is 6.2 or higher. It is common to blend 10% to 25% milk to neutralize acid in the whey and enhance yield and curd cohesiveness. The mixture is heated in a kettle to 82°C to 93°C (180°F to 199°F), followed by the addition of a food-grade acid (such as lactic, acetic, or citric) in quantity enough to drop the pH of the mixture to 5.9 to 6.1. In some plants, cultured milk and whey may be used as a source of lactic acid. The pH range is crucial to maintain the sweet flavor of the cheese. As a result of heat and acidity, the proteins denature and foam-type curd ascends to the surface.

The mixture is held for 15 to 20 minutes, after which the curd is dipped with a perforated ladle and collected in a muslin bag, which is allowed to drip and cool in a cold room. Alternatively, the curd is drained in perforated stainless hoops and allowed to dry. The curd is soft, fragile, and grainy, and may be pressed slightly to achieve cohesiveness. The yield of ricotta is low, around 5% to 6%, if no milk is mixed with the whey.

Mechanized production for continuous manufacture of ricotta cheese involves adjustment of the pH of the whey-milk mixture to 6.9 to 7.1 with caustic soda, heating the blend to 88°C to 92°C (190°F to 198°F), and injecting the appropriate quantity of salt and acid in-line. Again, the target pH is 5.3 to 5.5. The hot acidified whey-milk blend is pumped into the bottom of a V-shaped vat and the resulting curd is mechanically collected from the top of the vat into nylon mesh for the curd to drain. It is transferred to perforated hoops, cooled, and hot packaged for sale.

The ricottone cheese process is similar to that of ricotta, in that sweet whey is blended with whole milk, skim milk, or buttermilk. The pH of the blend is adjusted to 6.1 to 6.2 with a starter culture or edible acid. Ricottone curd is pressed and dried for four weeks at 21°C (70°F) to obtain dry ricotta cheese, a grating type of product.

Another variant of ricotta is mascarpone, which is made from cream (25% to 35% fat) by direct addition of citric or acetic acid to pH 5.0. Following drainage of whey in cloth bags, the cheese may be slightly salted, whipped, and formed into a cylindrical shape. Mascarpone has a creamier texture and rich flavor compared to ricotta. Unsalted mascarpone is used in the preparation of cakes and desserts such as tiramisu.

Impastata cheese is a starting material for pastry. It is made similar to ricotta, but the curds are allowed to sink to the bottom by

gentle stirring. The hot curd is drier than ricotta cheese. It is then ground into a smooth dough-like material for use in confectionery.

Process improvements have been accomplished by the use of ultrafiltration technology in ricotta cheese manufacture.

Chhana Cheese: Chhana is a ricotta-like product in the Indian subcontinent. It is obtained from hot milk by direct acidification. According to Indian Pure Foods Act (Aneja et al, 2002), chhana is defined as a product obtained from cow or buffalo milk or a combination thereof by precipitation with sour milk, lactic acid, or citric acid; it should contain no more than 70% moisture, and the milk fat content should not be less than 50% of the dry matter. Skim milk chhana is the product obtained from cow or buffalo skim milk by precipitation with sour milk, lactic acid, or citric acid; it should not contain more than 70% moisture, and the milk fat content of the product should not exceed 13% of the dry matter.

Chhana Cheese Process: Figure 11.5 illustrates the sequence of steps in the manufacture of chhana. The figure also shows the relationship among queso blanco, paneer, and chhana.

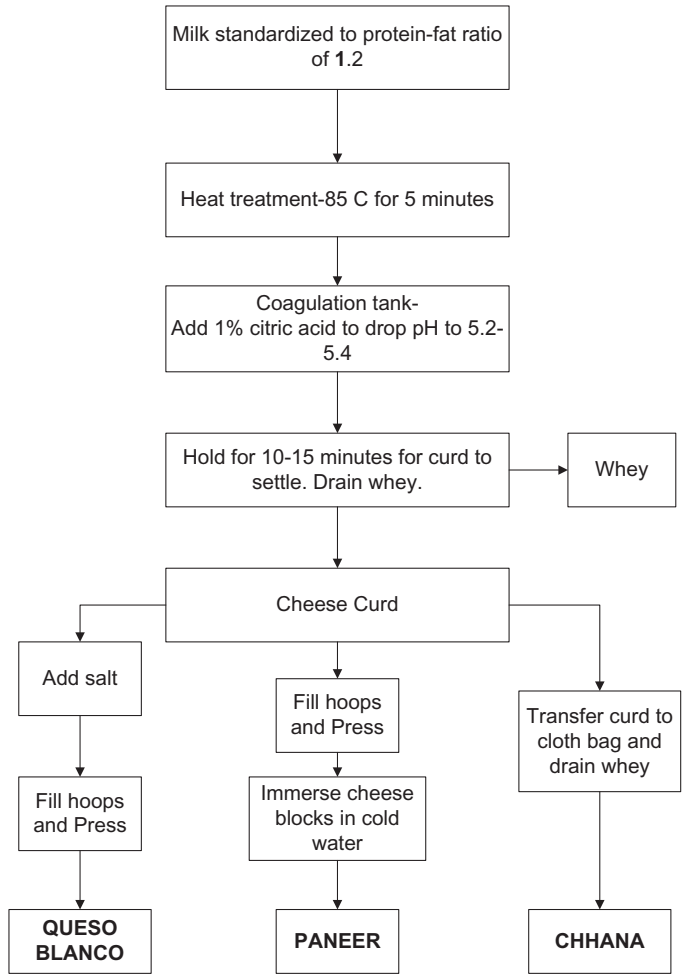

Figure 11.5. Flow sheet diagram for the manufacture of chhana, paneer, and queso blanco.

Cow's milk is preferred for making chhana, and the pressing of the curd is restricted to that obtained by gravity after draining of the whey in a cheesecloth/muslin bag. Longer draining time gives a hard product. Depending on its end use, chhana may be soft or hard. The texture varies from smooth and pasty to crumbly. For example, soft chhana is needed for the confection rasogolla, while the confection sandesh requires the hard variety. These two confections account for the majority of Chhana production, and optimum functionality is required in each case.

Chhana, like ricotta is made in kettles. Cow's milk is brought to near boiling and an appropriate acidulant is quickly added to the hot milk to bring the pH of the mixture to 5.4. The curd settles to the bottom and whey is removed by filtration through a strainer lined with cheesecloth. The curd is cooled in running tap water. Chhana is then used in sweetmeats and other Indian confections. The coagulants that are used are lime or lemon juice, vinegar, citric acid, lactic acid, fermented milk, and whey. A solution of citric acid (0.5% to 1.5%) or lactic acid (1% to 2%) is appropriate to lower the pH of hot milk to 5.4. The interaction between the temperature of coagulation and final pH affects the yield and quality of chhana. For cow's milk, a temperature of 80°C (176°F) at pH 5.4 is optimal, whereas for buffalo milk, 70°C (158°F) at pH 5.7 is desirable. A higher coagulation temperature imparts graininess and hardness to the texture, and lower temperatures result in sticky chhana, which drains significantly slower.

Paneer Cheese: Paneer, a non-melting cheese used for cooking and frying, is another acid-coagulated product of hot milk. It resembles chhana in its manufacture, but differs in that it is lightly pressed into blocks. It resembles tofu in appearance and texture, but possesses a distinct flavor of its own. Due to its ability to withstand cooking and frying temperatures, paneer offers outstanding non-melt functionality. This unique characteristic permits paneer's use in Indian cuisine, and it lends itself to the preparation of fried cheese snacks. The moisture content of paneer is no more than 70% and the fat content is at least 50% fat on dry matter basis. Skim milk paneer contains not more than 70% moisture and not more than 13% fat on dry matter basis.

Paneer Cheese Process: Paneer is typically made from buffalo milk in South Asia. It is made by heat and acid coagulation of the casein component of standardized milk. Through various interactions it entraps almost all of the fat, most of the denatured whey proteins and colloidal salts, as well as part of the soluble milk solids (in proportion to the moisture content retained). The typical chemical composition of paneer is 53% to 55% moisture, 23% to 26% fat, 17% to 18% protein, 2% to 2.5% lactose, and 1.5% to 2% minerals. Paneer is marble white with a slightly spongy body and close-knit texture. It has a mild sweetish-acidic nutty flavor.

The paneer production process is illustrated in Figure 11.5. The milk used for paneer should be standardized to a fat/solids-not-fat (SNF) ratio of 1 : 1.65 to achieve a minimum of 50% FDM in the finished product. Therefore, the starting milk must have a minimum of 5.8% milk fat. In India, buffalo milk or blended milk is preferred because it provides higher fat (6%) and SNF (9.5%) than cow's milk. In the West, the quality of paneer made from cow's milk is quite acceptable. Cow's milk is normally blended with 0.1% calcium chloride dihdrate to ensure good yield. High-quality paneer with 42% FDM can be obtained from milk that contains 3.5% fat and 8.5% SNF. Nevertheless, it is advantageous to standardize the milk to a protein/fat ratio of 1.2 with nonfat dry milk. Paneer has a soft, meaty texture and a pleasant, creamy flavor. Low-fat paneer is also available, with fat in the range of 24% FDM. Although skim-milk

paneer with 13% FDM is feasible, the product has a chewy, rubbery, and hard body.

Typically, milk is standardized to 3.9% to 4% fat and 13% SNF; low-heat nonfat dry milk is used to obtain the protein/fat ratio of 1.2. Calcium chloride is added at the 0.1% level. The milk is heated in a plate heat exchanger from 4°C (39°F) to 85°C to 90°C (185°F to 194°F) and pumped into a water/steam jacketed cheese vat and allowed to cool to 70°C to 75°C (158°F to 167°F) in about 10 minutes, prior to acid blending.

Many industrial processes use citric acid as a coagulant. Glacial acetic acid is also used by some manufacturers. The type and concentration of the acid and the mode of delivery and blending into the hot milk directly influence product yield and moisture retention. Citric acid monohydrate is generally used as 1% solution at 70°C (158°F). Generally, approximately 2 g of citric acid monohydrate is required to coagulate 1,000 g of milk. Sufficient acid is gently but quickly blended with the milk (within one minute) to bring about full coagulation. At this point, clear, greenish yellow whey separates out, allowing the curd to sink to the bottom. The pH of the whey should be 5.7 to 5.9. It is important not to stir the vat contents after coagulation to allow formation of large aggregates of curd.

Paneer obtained at a coagulation temperature of 70°C (158°F) has the best organoleptic quality as well as the most desirable frying quality in terms of shape retention, softness, and maintenance of integrity. Variation in the pH of coagulation has a profound effect on processing and quality parameters. For example, when the coagulation pH is increased from 5.1 to 5.4, the moisture content of paneer increases from 50% to 59% and the yield increases from 21% to 25%. Sensory quality is considered to be best at pH 5.30 to 5.35, the recommended pH for coagulation.

The coagulated milk is allowed to separate into curd and clear whey. After 10 minutes, nearly all of the curd chunks sink to the bottom. At this point, a strainer is fitted into the outlet of the cheese vat, the whey drainage valve is then opened, and the whey flows into a surge tank partitioned by a strainer to retain any curd fines escaping from the cheese vat. Hot whey accumulates in the second section of the surge tank from which it is pumped out, cooled to 4°C (39°F), and stored in a whey tank for further processing.

The curd is transferred to hoops lined with cheesecloth. The hoops have perforations on all sides to facilitate whey expulsion. Hoops may vary in size from 2 to 10 kg in capacity. Unsalted paneer is traditionally used for culinary purposes. Prior to placing the lid on the hoop and applying pressure, extra curd is added to facilitate block formation. The hoops are pressed with a hydraulic press to exert a low pressure (9.8 kPa) onto the curd. The pressing time is generally around 10 to 15 minutes, after which the wrapped blocks of paneer are ejected from the hoop for quick cooling. Moisture content, shear strength, and porosity of paneer is a function of the pressing conditions.

The wrapped blocks are cooled by immersion in cold water or 5% salt solution at 4°C (39°F) for two to three hours. The blocks absorb enough water during dipping to develop paneer's typical body and texture. Cooling also facilitates handling during cutting and wrapping for retail sale. Packaging materials include coextruded laminates of moisture barrier and oxygen barrier films, polyethylene sachets, and heat-induced shrink film. It is advantageous to freeze paneer packages for distribution over long distances and storage for extended periods.

Paneer yield ranges from 21 to 23 kg (containing 51% to 54% moisture)/100 kg of buffalo milk, corresponding to 63% to 67% milk solids recovery in the paneer. With cow's milk, the yield is around 17% to 18%. The process for making paneer has been

improved by the application of membrane technology (ultrafiltration).

Queso Blanco: This cheese is made from whole milk, skim milk, cream, or their mixture. The use of coagulating agents varies with the type of cheese. The production process involves either rennet coagulation of warm milk or curdling of hot milk with lime/lemon juice, fruit juice, or vinegar. Directly acidified cheese are include queso del pais, queso de la tierra, queso de cincho, and queso sierra. All are highly salted (salt level 2% to 4%) to improve their shelf life. Generally, Latin American white (LAW) cheeses are white, creamy in taste, highly salted, and acidic in flavor. They possess the body and texture of young, high-moisture cheddar and can be sliced for sandwiches.

Queso blanco made by direct acidification can be fried without melting, thus resembling paneer. The white cheeses can be used as a snack, in salads, as cooking cheese in casserole dishes, grated for use in pizza and other foods, or included as an ingredient in the manufacture of process cheese. LAW cheese is largely consumed fresh, but can be fried with or without butter to prepare nutritious snacks of excellent eating quality. The pressed cheese is hard and crumbly with a slightly open texture. Queso blanco typically contains 52% to 53% moisture, 22% to 24% protein, 16% to 18% fat, 2% to 3% lactose, and 2.5% salt. The pH is in the range of 5.3 to 5.5.

Queso Blanco Process: A typical manufacturing procedure for Queso blanco is shown in Figure 11.5. The procedure is similar to that of paneer, except for the addition of salt to the queso blanco curd. Furthermore, the blocks of pressed queso blanco are not immersed in cold water. Milk is fortified with nonfat dry milk to standardize it to a P/F ratio of 1.2. The milk is heated to 85°C to 95°C (185°F to 203°F) and held for 5 minutes to denature most of the whey proteins. Sufficient citric acid solution is pumped to the milk tank to drop the pH to 5.3 to 5.4. To coagulate 1 kg of hot milk, 2 to 2.5 g of citric acid monohydrate is required. Citric acid is used in the form of 1% to 1.5% citric acid solution heated to 70°C (158°F). The curd is allowed to settle in quiescent condition for 10 to 15 minutes. The whey is drained and the curd is trenched. After the curd is reasonably dry, it is dry-salted to reach the 2.5% salt concentration in the curd. The hoops are filled with hot curd and pressed at the pressure of 11 lbs/inch^2 (75 kPa) for three to four hours. After the hoops cool down overnight, the cheese blocks are removed and vacuum packaged in film. The yield of queso blanco is approximately 17 to 18 kg/100 kg milk. Queso blanco is ready for sale because it needs no ripening.

Process Cheese

Process cheese results from blending one or several natural cheeses of different ages with emulsifying salts, water, and other dairy and non-dairy ingredients. The mixture is heated with continuous agitation to produce a smooth pasteurized product that has an extended shelf life (Kapoor and Metzger, 2008). Walter Gerber and Fritz Stettler of Gerber and Co., Switzerland, invented process cheese in 1911 by heating Swiss cheese with a citrate-based emulsifying salt. In 1916 J. L. Kraft introduced process cheese in the United States (Zehren and Nusbaum, 2000). Kapoor and Metzger (2008) and Zeheren and Nusbaum (2000) extensively cover scientific and technological aspects of process cheese.

The CFR defines three major categories of process cheese based on the requirements for moisture, fat, pH, and the type and amount of allowed optional ingredients (21CFR 133.169 to 133.180) (CFR 2009a): pasteurized process cheese (PC), pasteurized process cheese food (PCF), and pasteurized process cheese spread (PCS). Table 11.12 summarizes the allowed ingredients and compositional specifications for PC, PCF, and PCS

Table 11.12. Major categories of process cheese produced and sold in the United States.

Category	Major ingredients and other optional ingredients (and their permitted levels)	Moisture (% w/w)	Fat (% w/w)	pH
PC[1] (21CFR133.169)	Cheese emulsifying salts (≤3% w/w of the final product) Acidifying agent Cream, anhydrous milk fat, dehydrated cream (weight of the fat derived is ≤5% w/w of the final product) Water, salt, colors, spices, flavors, enzyme-modified cheese, mold inhibitors (≤0.2 w/w or ≤0.3 w/w of the final product), anti-sticking agent (≤0.03 w/w of the final product)	≤40	≥30	≥5.3
PCF[2] (21CFR133.173)	Cheese (≥51% w/w of the final product) Other optional ingredients and their permitted levels include all of the ingredients allowed in PC in addition to milk, skim milk, buttermilk, and cheese whey	≤44	≥23	≥5.0
PCS[3] (21CFR133.179)	Cheese (≥51% w/w of the final product) Other optional ingredients and their permitted levels include all of the ingredients allowed in PCF in addition to food gums, sweetening agents, and nisin (≤250 ppm of the final product)	44–60	≥20	≥4.0
PCP[4] Substitute cheese/ imitation cheese	The amount of Cheese, the type and amount of other optional ingredients, and the compositional requirements are not defined; however, the final product should have organoleptic, physical, functional, and nutritional equivalence in terms of ingredients and composition to either PC, PCF, or PCS (21CFR 101.13(d) and 21CFR 130.10)	–	–	–

[1]PC, pasteurized processed cheese
[2]PCF, pasteurized processed cheese food
[3]PCS,pasteurized processed cheese spread
[4]PCP, pasteurized processed cheese product
Adapted from Kapoor and Metzger (2008)

made from cheddar cheese in the United States.

In addition to the above-mentioned categories, process cheeses also are presently sold under an additional category in the U.S. market, pasteurized process cheese product (PCP). PCP encompasses all the process cheese type products that do not meet the standard definition of PC, PCF, and PCS, either in terms of the ingredients added and/or the compositional specifications as described by CFR. PCP can further be subcategorized into substitute cheese and/or imitation cheese, respectively (21CFR 101.13(d) and 21 CFR 130.10) (CFR 2009b, 2009c) (Table 11.12). The nomenclature and labeling of PCP are still unclear and sometimes manufacturers may require special petitioning with the FDA. Substitute and imitation cheeses are discussed in detail later in this chapter.

Process Cheese Manufacture

Figure 11.6 indicates a schematic flow chart of process cheese manufacture. Process cheese manufacture involves two main stages:

1. Ingredient selection and formulation to prepare a homogeneous pre-blend
2. Cooking of the pre-blend to prepare a pasteurized, homogeneous semi-solid product followed by forming, packaging, cooling, and storage to produce the final process cheese

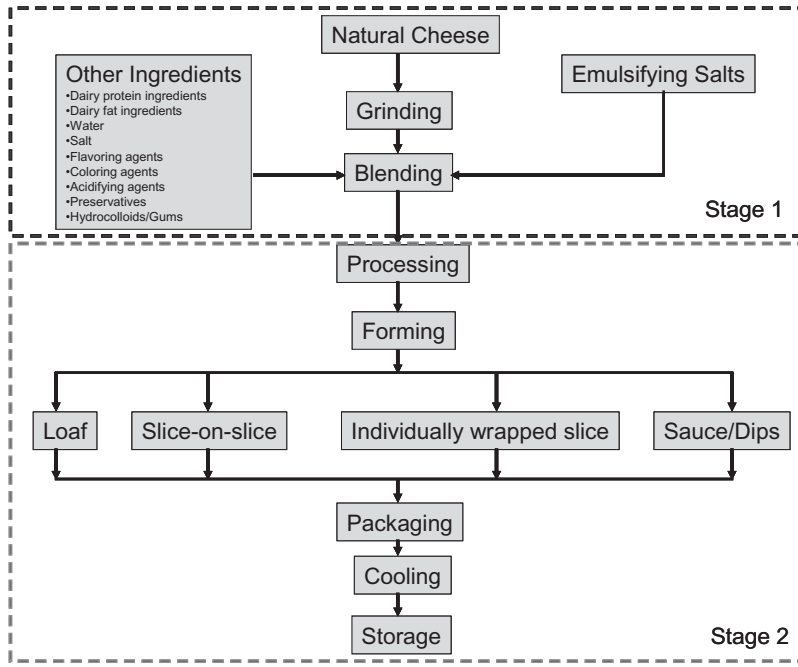

Figure 11.6. Manufacturing outline for process cheese.

The first stage involves selecting and grinding one or more natural cheeses, mainly on the basis of their age, pH, and flavor. This is followed by selecting the appropriate type and amount of emulsifying salts as described by the CFR (FDA 2008a). Other optional ingredients are selected to balance the target values for moisture, fat, salt, and pH of the final process cheese. All of the ingredients are then blended together to form a homogeneous pre-blend that is subjected to the second stage of process cheese manufacture.

Different process cheese manufacturers in the United States have different formulations, depending on ingredient availability as well as the type and the nature of the end use application for their products. Typical formulations for PC, PCF, and PCS are indicated in Table 11.13.

The second stage involves cooking (heating and mixing) the pre-blend prepared in the stage one to pasteurize it and form a uniform molten emulsion. This is followed by forming, packaging, cooling, and storage of the molten emulsified product to produce the final process cheese (Figure 11.6).

Process cheese manufacturers use a variety of cookers with different designs and operating conditions to manufacture process cheese. The cookers are designed for either batch or continuous production. They have various types of mixing and agitation systems as well as heating mechanisms (indirect heating or direct steam injection). Two common types of batch cookers are single or twin screw augers (Blentech Cooker, Blentech Corporation, Rohnert Park, CA) and high-speed cutting blade type cookers (Stephan Cooker, Sympak Inc., Mundelein, IL). The Rota Therm® continuous cooker (Gold Peg International Pty Ltd., Victoria, Australia) is gaining popularity in the process cheese industry for continuous process cheese manufacture. The most common method of heating in process cheese cookers

Table 11.13. Typical process cheese formulations for PC[1], PCF[2], and PCS[3].

% Ingredients	PC	PCF	PCS
Natural cheese[4]	80	71	60
Dried cream	6.1	–	–
Nonfat dried milk	–	3	4
Whey powder	–	2.9	3.6
Butter oil (anhydrous)	1.9	1.4	0.95
Whey protein concentrate	–	–	1.5
Emulsifying salt(s)[5]	2.25–2.50	2.25–2.50	2.25–2.50
Salt	0.5	0.6	0.8
Acidifying agent(s)[5]	–	–	0.3
Mold inhibitor[5]	–	–	0.2
Water	9	16.6	26.4
Total	100	100	100

[1]Pasteurized process cheese formula balanced to 39.5% moisture, 30% fat, and 2% salt
[2]Pasteurized process cheese food formula balanced to 43.5% moisture, 25% fat, and 2% salt
[3]Pasteurized process cheese spread formula balanced to 49.5% moisture, 20% fat, and 2% salt
[4]Natural cheese may be a combination of one or more cheeses with different degrees of maturity
[5]Ingredients may be one or a combination of the allowed ingredients for each of their respective categories as described by the CFR (21CFR 133.169 to 133.180)
Adapted from Kapoor and Metzger (2004)

is direct steam injection. Kapoor and Metzger (2008) have extensively described the operating conditions of various cookers that are used for process cheese manufacture.

After the molten process cheese is removed from the cooker it is subjected to forming and packaging processes to produce loaves, slices (slice-on-slice and individually wrapped slices), sauces, etc. Process cheese loaves are commonly used in both the retail and food service sectors. Slice-on-slice process cheese mainly is used in food service applications, whereas individually wrapped slices are primarily for retail sale.

In the case of process cheese loaf manufacture, aluminum foil or heat sealable plastic-lined cardboard boxes typically are filled with the molten process cheese. The quantity, depending on the type of market, varies from 30, 225-g consumer packs to two 5-lb bulk packs. As discussed above, process cheese slices are produced and sold in the United States as slice-on-slice or individually wrapped slices. The manufacture and packaging of sliced process cheese generally involves one or a combination of the following three methods: roller method, band or strip method, and injection method (Berger et al., 1998). The roller and band or strip method are common in the production of slice-on-slice process cheese. They involve a moving, cooling belt onto which the molten process cheese is poured and cooled. The cooled process cheese solidifies into a process cheese strip, which is removed from the belt with knives and cut into ribbons and slices. The injection method is commonly used for making individually wrapped slices; the molten process cheese is placed in preformed cylindrical tube-shaped packages, sealed, and then pressed in the form of slices (Berger et al., 1998).

Chemistry of Process Cheese

In contrast to natural cheeses such as cheddar, the microstructure of process cheese is a stable oil-in-water emulsion supported by a re-formed gel network of hydrated, emulsified caseins. Figure 11.7 describes the changes in the microstructure of process cheese during manufacture.

Adding emulsifying salts during process cheese manufacture enhances the emulsifica-

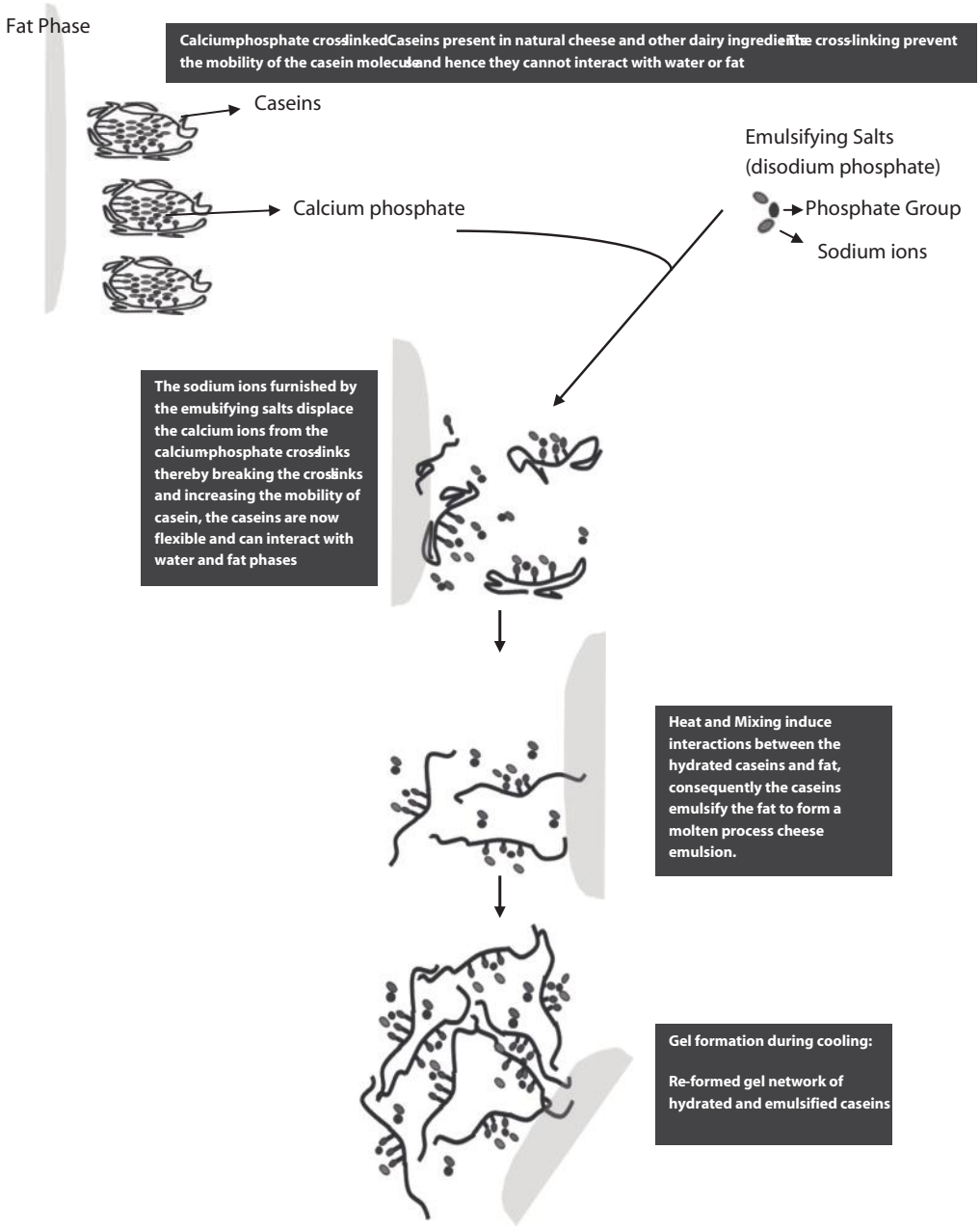

Figure 11.7. Changes in the microstructure of process cheese during manufacture. Adapted from Metzger (2004).

tion properties of caseins (derived from natural cheese and other dairy protein ingredients) by disrupting the calcium-phosphate cross-linked casein network. The disruption of this natural cheese casein network exposes the hydrophilic and hydrophobic sections of the individual caseins. Subsequently, when agitation and heat are applied, the disrupted caseins interact with the aqueous phase via hydrophilic interactions. Consequently, the hydrated caseins also emulsify the fat phase via hydrophobic interactions. These interactions cause the fat phase to be emulsified by a uniform closely knit protein network. Ingredients (including the type, source, and age of the natural cheese used; type and amount of emulsifying salts; and other ingredients used while formulating a process cheese) and processing conditions (cook temperature, cook time, rate of mixing during cooking, and the rate of cooling after manufacture) are very important in providing the final process cheese with its unique microstructure. This unique microstructure provides process cheese with variety of functional properties such as un-melted and melted texture that can be engineered in different ways to achieve a product with custom end use functionality. Process cheese is used in a variety of food applications such as pizza, burgers, frozen and shelf-stable entrees, dips, sauces, soups, etc. in both the retail and food service sectors of the food industry.

Effect of Formulation Parameters and Ingredients on Process Cheese

Table 11.14 describes the various formulation parameters, their influence on the final quality of process cheese, and formulation suggestions to achieve the desired process cheese. Table 11.14 also describes the various ingredients that contribute to these formulation parameters. Details and the effects of some of the major ingredients are indicated below.

Natural Cheese

Natural cheese is the major ingredient used in a process cheese formula. Based on the type of process cheese manufactured (Table 11.12), natural cheese can vary from 51% to almost 85% of the final process cheese. Appropriate selection of natural cheese is very important to render desired final process cheese. Typically, natural cheese (one or a combination) is selected according to its type, flavor, age, texture, and pH. Manufacturers use subjective (sensory) and analytical techniques to select the desired natural cheese for manufacturing process cheese. As indicated in Table 11.14, natural cheese has an influence on the pH, intact casein, and total calcium content of the final process cheese.

Emulsifying Salts

Emulsifying salts are ionic compounds composed of monovalent cations and polyvalent anions. They play a major role in the formation of the process cheese gel network (Figure 11.7). The two main functions of emulsifying salts are:

1. Chelation of calcium (that aids in breaking the calcium-phosphate cross-linked protein network present in natural cheese).
2. pH adjustment

Both functions help to engineer the appropriate protein interactions during process cheese manufacture (Figure 11.7) to achieve the desired process cheese gel network and consequently the required functional properties such as meltability and unmelted texture. Therefore, appropriate selection of the type, quantity, and combinations of emulsifying salts can help in achieving the desired final functionality.

CFR defines 13 emulsifying salts that are allowed (singly or in combination) in process cheese (21CFR 133.169 to 133.180).

Table 11.14. Effect of various formulation parameters on the functionality and quality of process cheese.

Formulation parameter	Description and effect on final functionality/quality	Optimum/typical value	Ingredients contributing to the parameter*	Formulation suggestions
Moisture content	Directly related to meltability Inversely related to firmness	Typically defined by the regulations and/or the end user specifications	Natural cheese Added water	Not very critical in controlling the functionality of process cheese due to the regulations Gain importance for non-standard cheese products such as analogs
Fat content	Directly related to meltability Inversely related to firmness	Typically defined by the regulations and/or the end user specifications	Natural cheese Added fat (dried cream, liquid cream, anhydrous milk fat, butter oil etc.)	Not very critical in controlling the functionality of process cheese due to the regulations Gain importance for non-standard cheese products such as analogs
pH	Affects the protein interactions and therefore the final gel network of process cheese pH < 5.4 weakens the gel network; hence, final cheese is softer and crumbly Higher pH (approx. 6.0) makes the cheese tougher, difficult to process	5.6 to 5.8	Natural cheese Emulsifying salts Re-work pH controlling agents (acidulants)	Appropriate selection of type and amount of emulsifying salts to achieve the desirable pH Appropriate selection of natural cheese Use of acidulants to achieve the desired pH
Intact casein content	Refers to the amount of un-hydrolyzed protein in the process cheese formula Affects the protein interactions and therefore the final gel network of process cheese Higher intact casein reduces the meltability and increases the firmness of the process cheese	12 to 16% (selection based on the product type and final functionality desired)	Natural cheese Other ingredients contributing to dairy protein	Select younger natural cheese for a higher intact casein level and vice versa Appropriate selection of allowed dairy protein powders that contribute to intact casein Typically all the casein contributed by dairy powders is intact casein
Total calcium content	Affects the protein interactions and therefore the final gel network of process cheese Higher total calcium content makes the cheese difficult to process, reduces the meltability, and increases the firmness	Selection based on the product type and final functionality desired	Natural cheese Other dairy ingredients contributing to dairy calcium	Natural cheese selection based on calcium content desired in the final process cheese Appropriate selection of type and amount of emulsifying salts to achieve desired calcium chelation during process cheese manufacture consequently reduces the amount of calcium available for protein interactions
Lactose content	Higher lactose to moisture ratio can lead to formation of glass-like lactose crystals (a quality defect) Higher lactose content may lead to Maillard browning, thereby affecting the final flavor and color of process cheese	Typical lactose to moisture ratio in process cheese should not exceed 14%.	Dairy ingredients contributing to lactose (whey powder, nonfat dried milk, whey protein concentrate) Re-work	Formulate the process cheeses within the desired lactose to moisture ratio
Whey protein content	Affects the gel network by forming heat induced irreversible gels Higher levels reduce the meltability and increase the firmness	Selection based on the product type and final functionality desired	Dairy ingredients contributing to whey protein (whey protein concentrate, nonfat dried milk)	Formulate with appropriate selection of ingredients to achieve desired level of whey protein

*Details of effects of some of the major ingredients are discussed in the text

Table 11.15 describes the salient features of some commonly used emulsifying salts in process cheese.

Re-work

Re-work is another ingredient, typically overlooked by manufacturers, that has a marked influence on the final functionality of process cheese. Re-work is the process cheese produced in a manufacturing facility that cannot be sold for various reasons and hence for economic reasons finds its way back into fresh batches of process cheese during production. It can further be classified as old and fresh re-work. Old re-work refers to the process cheese that failed to meet quality guidelines (mechanical, weight related, or chemical) and therefore cannot be sold and typically is not used immediately. Fresh re-work is the process cheese lost (and collected) during production line change-overs, shavings, and edge trimmings during slice line operations etc. and is therefore typically used immediately in the subsequent batches. Because the re-work has already gone through the emulsification process, its addition to fresh batches of process cheese can alter their functional properties, flavor, and color. The addition of re-work to process cheese typically increases its firmness and reduces its meltability. Typical usage of re-work in process cheese should not exceed 1% to 2% of the final formula in the case of old re-work or not more than 10% in the case of fresh re-work (Kapoor and Metzger, 2008).

Effect of Processing Conditions on Process Cheese

The temperature and time of cooking, amount of agitation provided during cooking, and the rate at which the cooked process cheese is cooled affect the functional properties of process cheese. Therefore, varying the processing conditions during the manufacture can be another tool to engineer process cheese with targeted final properties. Table 11.16 describes the various processing conditions and their influence on the final quality of process cheese.

Cold-pack cheese is yet another class of cheese ingredient defined by the CFR that can neither be directly classified as a natural cheese nor as a process cheese. Cold-pack cheese is increasing being used as a cheese ingredient, especially for flavor in the manufacture of cheese sauces and dips for frozen entree preparation.

Cold-pack or Club Cheese

Cold-pack cheese, or club cheese, is prepared by comminuting one or more cheeses into a homogeneous mass without the aid of heat. Cream cheese, Neufchatel cheese, cottage cheese, hard grating cheese, semi-soft part-skim cheese, part-skim spiced cheese, and skim milk cheese for manufacturing are not permitted. All cheeses used in a cold-pack cheese are made from pasteurized milk or are held for not less than 60 days at a temperature of not less than 1.7°C (35°F) before being comminuted. No water is used in the preparation of the cold-pack cheese. The fat content of the product made from a single variety of cheese is not less than the minimum for the variety of cheese used, but in no case is less than 47% FDM. In the case of cold-pack Swiss cheese, the fat content is not less than 43% FDM, and in cold-pack gruyere cheese it is not less than 45% FDM. The moisture content of a cold-pack cheese made from two or more varieties of cheese is not more than the arithmetical average of the maximum moisture contents of the varieties of cheese used. In no case is the moisture content more than 42%, except that the moisture content of a cold-pack cheese made from two or more varieties of cheddar, washed-curd, colby, and granular cheese is not more than 39%. The FDM of a cold-pack cheese made from two or more varieties of is not less than the arithmetical average of the minimum percentage

Table 11.15. Salient features of certain commonly used emulsifying salts in process cheese.

Emulsifying salt	Chemical formula	pH, 1% solution	Calcium chelation power	Effect on firmness of process cheese*	Effect on meltability of process cheese*	Other properties and typical products used in
Trisodium citrate (dihydrate)	$Na_3C_6H_5O_7.2H_2O$	8.6	Low	2	4	Typically used in slice-on-slice process cheese
Disodium phosphate (dehydrate)	$Na_2HPO_4.2H_2O$	9.1	Low	2	2	Used in loaves and cheese spreads Has bacteriostatic properties, used in shelf-stable sauces and process cheeses
Trisodium phosphate (dodecahydrate)	$Na_3PO_4.12H_2O$	11.9	Low	1	2	Mainly used in conjunction with other emulsifying salts Used for pH adjustment Has bacteriostatic properties
Sodium hexametaphosphate	$(NaPO_3)_n$ (n=10–15)	6.6	High	3	1	Used in conjunction with other emulsifying salts Commonly used to manufacture reduced melt/no melt process cheese Has bacteriostatic properties
Sodium aluminum phosphate		9.2	Low	3	3	Commonly used in imitation mozzarella-type cheese analogs

*The numbers have no numerical significance. They simply indicate the relative order of the effect of salts on the specific process cheese property with the assumption that the each of the salts is used at the same level. A higher number indicates higher values of the described property. Please note that the amount used as well as combinations of the above indicated salts lead to different properties

Adapted from Kapoor and Metzger (2008) and Fox et al., (2000b)

Table 11.16. Effect of processing conditions on the functionality of process cheese.

Processing condition	Description and effect on final process cheese functionality and quality
Cook temperature	Higher cook temperature reduces the meltability and increases the firmness, and vice versa
Cook time	Longer cooking time reduces the meltability and increases the firmness, and vice versa
Amount of agitation during cooking	Increased agitation reduces the meltability and increases the firmness, and vice versa
Rate of cooling	Slower rate of cooling reduces the meltability and increases the firmness, and vice versa

of fat of the varieties of cheese used, but in no case is it less than 47%, except for Swiss cheese and gruyere cheese, which is not less than 45%.

The weight of each variety of cheese in a cold-pack cheese is similar to that as in pasteurized process cheese. The pH of cold pack cheese is adjusted with vinegar, lactic acid, citric acid, acetic acid, or phosphoric acid to 4.5 or above. The other ingredients are water, salt, color, spices, and flavorings.

Cold-pack cheese in consumer-sized packages may be preserved with sorbic acid, potassium sorbate, sodium sorbate, (maximum level 0.3%), or sodium propionate and/or calcium propionate (maximum level 0.3%).

Cold-pack Cheese Food

Cold-pack cheese food is similar to cold pack cheese, except the moisture content is not more than 44% and the fat content is not less than 23%. The weight of the cheese ingredient constitutes not less than 51% of the weight of the finished cold-pack cheese food. Pasteurized dairy ingredients (cream, milk, skim milk, buttermilk, whey, anhydrous milk fat, dehydrated cream, skim milk cheese for manufacturing) are permitted in cold pack cheese food. Sweeteners (sugar, dextrose, corn sugar, corn syrup, corn syrup solids, glucose syrup, glucose syrup solids, maltose, malt syrup, and hydrolyzed lactose) also can be used. In the preparation of cold-pack cheese food, guar gum or xanthan gum, or both, may be used, but the total quantity of such an ingredient (or a combination) is not to exceed 0.3% of the weight of the finished food. When one or both such optional ingredients are used, dioctyl sodium sulfosuccinate may be used in a quantity not in excess of 0.5% by weight of the gums. The product may contain preservatives as in cold pack cheese.

Cold-pack cheese food can contain particulates of fruits, vegetables, or meats. All cold cheese products are made by thorough grinding and mixing in a cheese blender similar to a meat blender used in sausage manufacture.

Cheese Substitutes/Analogs

Several cheese substitutes are now available in the United States, including cheddar, mozzarella, Swiss, colby, gouda, provolone, process, cream cheese, and cheese spreads. They offer ingredient cost savings, special functional properties (restricted melt), and desirable dietary attributes (cholesterol free, reduced saturated fat). The food and drug regulations may require them to provide equivalent nutrition to real cheese (Table 11.12). Accordingly, they may be fortified with vitamins and minerals. They are basically classified as:

- Filled cheeses, in which vegetable oils and/or some milk fat are emulsified in skim milk before normal cheese making. Filled cheeses are low in cholesterol and lower in saturated fatty acids, and thus are used in medically prescribed diets.

Table 11.17. Some formulations for cheddar cheese substitutes and pizza cheese substitutes.

Cheddar cheese substitute		Pizza cheese substitute	
Ingredients	% by weight	Ingredients	% by weight
Sodium and calcium caseinate	22	Caseinates	22
Soy oil	24	Soy oil	24
Lactic acid	1	Starch	2
Starch	1	Emulsifying salts	2
Emulsifying salts	1.5	Flavors	3.5
Flavor/flavor concentrates	1.5	Stabilizers	0.50
Stabilizer/emulsifier	0.5	Lactic acid	0.40
Salt	1.5	Color	0.04
Water	34	Preservatives	0.10
Cheddar cheese	13	Water	45.46
Total	100	Total	100

Adapted from Hill (2009), Guinee (2003)

- Cheese analogs, which may be formulated with non-dairy ingredients such as soy protein, soy oil, flavors, emulsifiers, and stabilizers. Some analogs may be partial dairy and include casein, soy oil, flavors, emulsifiers, and stabilizers. Another analog includes protein and fat derived from casein and anhydrous milk fat along with emulsifiers and stabilizers. They are designed to reduce costs and provide certain perceived nutritional advantages over regular cheese. Some analogs may contain soy protein in place of caseins.

A typical formulation of cheddar cheese substitute is given in Table 11.17.

In general, cheese substitutes are formulated with rennet casein and caseinates of sodium and calcium to impart stretchability, shreddability, melting properties, and simulated texture. In some cases starch replaces casein for economic reasons. Sodium phosphates and citrates are used as emulsifying salts to melt the ingredients into a homogeneous mass at cooking temperature 85°C (185°F). In addition, guar gum, xanthan gum, and carrageenans assist in moisture management, resulting in desirable texture and product stability. Natural cheese, enzyme-modified cheese, starter distillates, glutmates, or yeast autolyzates are blended in for flavor adjustment. Permissible colors are added to impart desirable color. Sorbates, nisin, and propionates are used to extend shelf life. Citric or lactic acid is used for pH adjustment of the final product. The substitutes are fortified with minerals and vitamins such as calcium oxide, magnesium oxide, zinc oxide, iron, vitamin A, riboflavin, thiamin, and folic acid because federal regulations require substitutes to furnish nutrients equivalent to regular cheeses.

The attributes of regular cheese and their substitutes are not necessarily identical, as shown in Table 11.18. The manufacturing flow diagram for cheese substitutes is illustrated in Figure 11.8.

Applications in Foods

Cheeses are used in food products for their nutritive merits, flavor, and texture. Cheese for manufacture of food products may offer economical opportunities because it does not necessarily have to meet the specifications for more expensive retail grade. Natural cheese is ripened, cured, or aged at a certain temperature (and humidity in some cases) for various periods to develop flavor and texture characteristics of particular cheeses. The different cheese varieties offer unique attributes when used in food applications. Typical ripening periods used in the industry are given in Table 11.19.

Table 11.18. Examples of proximate composition of low-moisture, part-skim mozzarella (pizza cheese), cheddar cheese, and their analogs.

Component	Low- moisture, part-skim mozzarella cheese	Low-moisture, part-skim mozzarella cheese analog	Cheddar cheese	Cheddar cheese analog
pH	5.2	5.8	5.3	5.8
% Moisture	48.5	51.5	37.5	52.0
% Protein	26.3	16.0	23.8	16.5
% Fat	21.0	22.5	32.5	23.5
% Fat in dry matter	37.9	46.39	52.5	48.9
% Salt	1.8	0.8	1.7	1.7

Adapted from Guinee (2003)

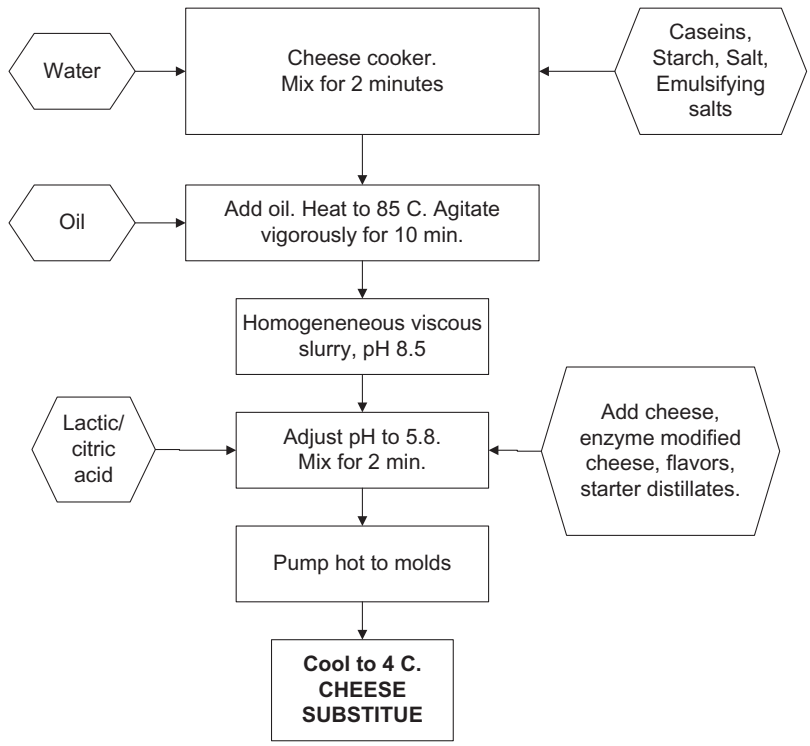

Figure 11.8. Flow diagram for cheese substitute (analog) manufacture.

The characteristic flavor of certain cheeses provides versatility in culinary dishes, food service items, easy-to-prepare meals, dry snacks, and formulated foods. Popular culinary dishes made from uncooked cheese include cheesecake, desserts, and salads. The cooked dishes include cheese-filled meat, fish, and vegetable dishes; fried cheese curd; fried paneer dishes; paneer curries; fondues; cheese omelettes; pizzas; pasta; quiche; and many other commonly consumed dishes. Consumer foods with cheese ingredient include au gratin potatoes; mashed potatoes; cheese-filled bread; fried, batter-enrobed mozzarella sticks; cheese-filled vegetables, meats, and fish; prepared meals; refrigerated and frozen pizza; cheesecake; cheeseburgers; fries, and cordon blue entrees. In addition, cheese-coated extruded grain snacks, cheese crackers, infant meals, Italian meals, soups,

Table 11.19. General guidelines for ripening periods of various cheeses.

Cheese variety	Ripening period
Blue	2 months minimum
Brick	2 weeks
Cheddar	2 weeks to 12 months
Colby	2 weeks to 3 months
Cream cheese	No ripening
Gouda	3 to 6 months
Monterrey Jack	2 to 6 months
Mozzarella	Unripened to 3 weeks
Muenster	2 to 8 weeks
Parmesan	10 months minimum
Provolone	2 to 12 months
Romano	5 months minimum
Swiss	2 to 9 months

dips, sauces, and fillings are manufactured using cheese as an ingredient. In this regard, natural cheese, process cheese, dry cheese, cheese sauce, and enzyme-modified cheese have been developed with specific functional attributes which facilitate the use of cheese in unheated or cooking form.

The flavor profile of cheeses is related to their use in various foods, as shown in Figure 11.9. Cheese imparts a desirable background flavor and concomitantly acts as a flavor carrier of spices and herbs in the food.

The textural attributes of cheeses play an important role in their applications. To facilitate their use, various cheeses must be converted to usable form. Certain cheeses lend themselves to better use than others because of their functional characteristics. The ability to form shredded strips of uniform length, width, thickness, and resistance to clumping during shredding is a unique attribute of cheeses such as low-moisture mozzarella, process cheese, medium cheddar, gouda, and provolone, thus permitting their convenient use in heated applications such as pizza, Mexican dishes, Italian dishes, grilled cheese sandwiches, etc. Some cheeses possess the ability to form thin slices without breakage to allow their use as inserts in cold and grilled sandwiches, as exemplified by low-moisture mozzarella, Swiss, provolone, and process cheese.

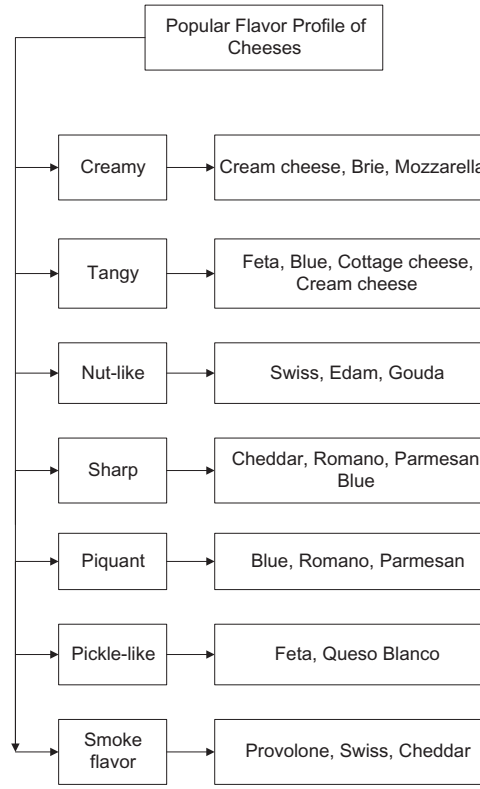

Figure 11.9. Popular cheese flavor profile related to their applications in foods.

Another attribute is some cheeses' ability to grate easily into small particles for use as sprinkles in Italian cuisine, such as Parmesan and Romano cheeses. Feta and blue cheeses are crumbly, which makes their application in salads very appealing. Cream cheese, process cheese foods and spreads, cold-pack cheese products, well-ripened Camembert, and brie cheeses exhibit unique spreadability characteristics. String cheese has a desirable property to form long strands on peeling. Mozzarella and other pasta filata cheeses demonstrate desirable stretchability on heating, as in pizza.

The performance of some cheeses on heating is specifically associated with applications in foods. Figure 11.10 shows examples of cheese ingredients whose performance

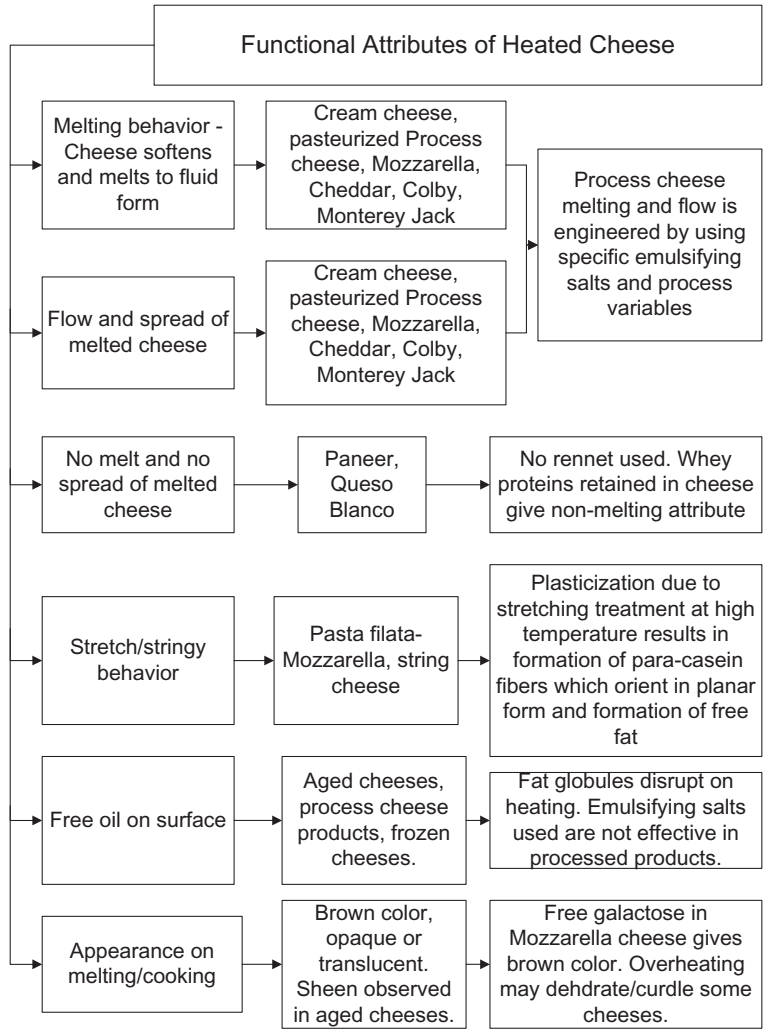

Figure 11.10. Functional attributes of cheese upon heating.

is significantly influenced by their functional attributes.

These attributes may be accentuated by formulation and processing variables. Cheese products with retarded melting and flowability fulfill a demand in meals in which cheese melting must be restricted. On the other hand, cheese slices are formulated to furnish quick melting, as is desired in fast food operations. If cheese with no melting attribute is required for frying, paneer and queso blanco cheeses are ideal. Thus, the cheese industry can design products according to the food manufacturer's needs.

Typical attributes and uses of various cheeses in foods are summarized in Table 11.20.

Hispanic cheeses are becoming popular and more economically significant. Their attributes and uses are given in Table 11.21.

Finally, some common problems associated with the applications of cheese, their causes, and solutions are enumerated in Table 11.22.

Table 11.20. Popular cheese varieties, their characteristics and uses.

Cheese	Color	Flavor	Body	Texture	Common uses
Cheddar	Creamy white to dark orange	Mild to sharp	Firm	Smooth	Appetizers, crackers, sandwiches, snacks, pizza, salads, vegetables, pasta, entrees, breads, cooking, fondues, sauces
Colby	Creamy to deep yellow	Mild	Medium firm	Slightly open	Appetizers, snacks, salads, sandwiches
Brick	Creamy yellow	Mild to sharp	Medium firm, semi-soft	Smooth, waxy	Appetizers, snacks, sandwiches, served with fruit desserts
Muenster	Creamy white	Mild	Semi-soft	Smooth	Nibbling, appetizers, snacks, sandwiches, fruit desserts
Blue	Blue-veined white	Piquant	Crumbly	Pasty	Salads, salad dressings, appetizers
Mozzarella	Creamy white to white	Delicate mild	Soft	Plastic	Pizza, lasagna, sandwiches, snacks
Gouda	Creamy white	Mild, nut-like	Semi-soft	Smooth with small eyes	Sandwiches, cooked dishes, appetizers, salads, with fruit as dessert
Edam	Creamy white	Buttery, mild, nutty	Semi-soft	Smooth with small eyes	Sandwiches, salads, cooked dishes, with fruit as dessert
Monterrey Jack	Creamy white	Mild, creamy	Semi-soft	Smooth	Sandwiches, cheese trays, Mexican foods, flavored with hot pepper and spices
Provolone	Light yellow	Salty, mild to sharp	Semi-hard	Smooth	Snacks, appetizers, ravioli, lasagna, desserts, Italian foods
Parmesan	Creamy white	Mild	Very hard	Granular	Grated for use in lasagna, spaghetti, pizza, breads, soups, salads
Romano	Yellowish white	Sharpe	Very hard	Granular	Grated for use in lasagna, spaghetti, pizza, breads, soups, salads
Swiss	Creamy white	Nutty, sweet	Hard	Smooth with large eyes	Sauces, fondues, appetizers, sandwiches, soups, desserts
Port du Salut	Creamy yellow	Robust, full flavor	Semi-soft	Creamy	Appetizers, dessert cheese with wine, fruits and sweets, and crackers
Bel Paise	Creamy	Slightly tart, lingering flavor	Semi-soft	Creamy soft	Appetizers, dessert cheese with wine, fruits and sweets, and crackers
Limburger	Creamy white	Aromatic, strong flavor	Soft	Waxy	Served with dark breads, snacks, appetizers, with fruit desserts
Feta	White	Salty, piquant	Soft	Creamy soft	Salads, Greek foods, Middle Eastern foods
Cottage cheese	White	Creamy	Soft, unripened	Curd particles (large/small)	Salads, dips, in cooking, Blintzes
Cream cheese	Creamy white	Mild, delicate	Soft, unripened	Buttery	Crackers, cheese balls, bagel spreads, salads, dips, toppings, sandwich spreads, cheesecake, desserts
Ricotta	Creamy white	Mild, cooked	Soft, smooth paste	Smooth, pasty	Salads, dips, lasagna, ravioli
Paneer	Creamy white	Dairy, creamy	Soft, softens on heating but does not melt	Sliceable, can be diced	Curry dishes, fried enrobed snacks, appetizers

Adapted from Chandan (1997), Guinee (2003)

Table 11.21. American Hispanic cheeses and their characteristics.

Category	Name	Flavor	Texture	Applications
Fresh, non-melting type	Queso blanco	Mild, creamy	Grainy, curdy	Shredded and sliced for use as topping and filling in cooked dishes
	Queso fresco	Mild, milky, salty	Crumbly, moist	Grated for use in cooked dishes and for garnishing
	Queso para freir	Mild, creamy	Firm, moist	Sliced and shredded, deep-fried, pan fried; crumbled for sprinkling on beans, salads, fruit
	Queso requeson	Fresh milky	Grainy, ricotta-like	Crumbled and used as a filling in cooked foods, desserts, and sprinkled on salads and soups
	Queso panela	Mild, sweet, milky	Firm, moist, mozzarella-like	Sliced and shredded for sandwiches, salads, cooked dishes
	Queso crema	Mild, sweet, creamy	Firm, moist	Shredded and sliced for sandwiches, salads, cooked dishes
Melting type: melts to smooth texture	Queso Asadero	Mild, tangy, provolone-like	Firm	Sliced and shredded for quesadillas, grilled sandwiches, nachos, hamburgers
	Queso Oaxaca	Mild, salty, sweet milk-like	Firm	Shredded for use in cooked dishes
	Queso menonita/ Chihuahua/quesadilla	Mild	Smooth, gouda-like	Sliced and shredded for sandwiches and as table cheese
	Queso manchego	Nutty, mellow	Firm	Sliced and shredded for sandwiches, and as a snack with wine and fruit
Hard, aged type	Queso cotija	Very salty, piquant	Hard, dry, feta-like, crumbly	Grated for use in cooked food, soups, and for garnishing beans, salads
	Queso cotija anejo	Very salty, pungent	Dry, hard, crumbly	Grated and crumbled for use in salads, cooked foods
	Queso duro	Salty, smoky/ fishy	Firm, dry	Crumbled for use in quesadillas, queso fritos, and on beans
	Queso enchilado	Salty, paprika-spicy	Hard, dry, crumbly	Grated and crumbled for use in salads, soups, and hot foods

Adapted from Dairy Management, Inc. (2007)

Table 11.22. Troubleshooting guide for cheese applications.

Problem	Reason	Solution
Not enough flavor	Cheese is either served cold or is not aged enough and needs additional time for flavor development	Serve cheese at room temperature or replace with more aged cheese
Cheddar cheese does not shred.	Cheese has developed a short body	Change to a fresher cheddar cheese, shred at lower temperature
Cheese looks greasy	Oiling off.	Cheese has been left out at room temperature, store cheese covered and refrigerated
Natural cheese does not melt properly	Different natural cheeses have differing melting tolerances	Check oven settings and time exposures or try different ages of natural cheese
Cheese is dried out and hard	Cheese has been left out uncovered.	Grate dried out cheese, use for topping or flavoring in casseroles
Cheese is very difficult to slice	Cheese is soft and pliable	Cheese should be sliced cold, higher moisture natural cheeses have different slicing characteristics than lower moisture cheese, adjust to thicker slice
Cheese has mold growing on it	Moisture and air exposure has caused mold to grow	Store cheese in a tight, moisture-proof wrap or bag; the cheese interior is acceptable—scrape off mold and 1/2 inch of subsurface
Cheese is crumbly and mealy	Cheese has been frozen	If freezing is necessary, freeze in unopened original wrapper or rewrap in small pieces of moisture-proof wrapping and freeze immediately, thaw in refrigerator, use crumbly cheese in cooking instead of slices
Process cheese does not melt or melts too fast	Too much heat or not enough heat, use correct type of process cheese	Check oven temperature thermostat, use specially formulated cheese with retarded or melting characteristics
Loaf cheese wrappers stick to the cheese	Storage too cold	Ideal temperature to store cheese is 38°F to 42°F, warm the cheese to room temperature

Adapted from Chandan (1997), DMI (1997)

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Chapter 12

Enzyme-modified Dairy Ingredients

Arun Kilara and Ramesh C. Chandan

Introduction

Enzymes are biocatalysts. Catalysts speed up reaction rates without undergoing permanent changes, and biological catalysts are important in all life processes. It is an axiom that life itself would not exist if not for enzymes. The operating characteristics of enzymes include substrate specificity, optimum temperature, optimum pH, and other environmental factors. Almost all known enzymes are proteins and range in size from 13,000 to several million Da. Only a few residues within a structure of the enzyme are important for the catalytic activity.

Commercial enzymes are derived from animal, plant, and microbial sources. Although the International Union of Biochemists recommends scientific classification of enzymes, colloquial names predominate in the food industry. Most enzyme names end in "–ase" and are derived from the source of the enzyme or the substrates which they act on. Hence, enzymes acting on proteins are called proteases, those acting on lipids, lipases; and carbohydrates, carbohydrases. Among proteases, enzymes derived from plants such as those from papaya (*Carica papaya*) are called papain; from fig latex, ficin; or from pineapples, bromelain. Clearly, these proteases do not end in "ase," reflecting the vagaries of colloquial nomenclature of enzymes. Animal-derived enzymes come from the organs of domesticated farm animals; trypsin, chymotrypsin, pre-gastric lipase, pancreatin, and the like are commonly isolated from porcine or caprine sources. Both animal and plant sources of enzymes impose severe constraints on the production of large quantities of enzymes. Therefore, with the advent of biotechnology, microbial enzymes prevail.

Microbial enzymes for commercial use are extracted from yeasts, molds, or bacteria. They are contained within the cells or secreted into the growth medium. Enzymes contained within the cells are termed endocellular, whereas those secreted into the growth medium are extracellular. Extracellular enzymes are generally low-molecular-weight proteins (30 to 50 kilodaltons) and contain disulfide bridges. Endocellular enzymes regulate the metabolism of organisms (regulatory enzymes) and are of higher molecular weights, and often their structures involve subunits.

Materials on which enzymes act are called substrates. Those of interest in the dairy industry are proteins, lipids, and lactose. As detailed in Chapter 2, milk proteins are classified as caseins and serum proteins. Milk fat consists of triglycerides and a number of other lipid substances. Lactose is the main carbohydrate in milk.

Enzymes transform fluid milk to products. For example, treating milk with the enzyme lactase yields low-lactose milk suitable for consumption by people unable to digest

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lactose. The enzyme chymosin, or rennet, is a key to the manufacture of cheeses and gelled dairy products. Microbes that contain enzymes transform milk to yogurt, and cream to sour cream and cream cheese. In these microbial transformations, the lactose content of products is reduced and desirable textures are created. Various chapters in this book discuss microbially transformed dairy products.

Enzyme-modified proteins and lipids are another category of dairy ingredients. Although dairy ingredients are relatively expensive to use in cost-sensitive formulations, in some instances, modification of functionalities of ingredients is desirable and enzymes are useful for this purpose. For example, enzymes help to obtain the nutritionally desirable fractions of macromolecules that have recently become popular additions to foods.

This chapter discusses protein and lipid modifications in dairy ingredients. Lactose modification is not discussed because it is used only in fluid milk products (beverage milk).

Protein Modification

Proteins are increasingly being used to perform functional roles in food formulations. Common food proteins have good functional properties including solubility, gelation, emulsification, and foaming. The functional properties of proteins are impaired near their isoelectric point (pI), as is the case in most acidic foods. Enzymatic modification of food proteins by controlled proteolysis can enhance their functional properties over a wide pH range and other processing conditions.

Choosing the right proteolytic enzyme, environmental conditions for hydrolysis, and degree of hydrolysis is crucial for enhancing the functional properties of proteins. Understanding the molecular properties required for protein functionality and the development of strategies to achieve them is critical for developing and using modified protein ingredients. Numerous reports on enhancing the functionality of food proteins by limited proteolysis have been published. Functionality imparts a valuable dimension to food proteins, complementing their established nutritional value.

The functional properties of proteins are those physicochemical properties that govern their performance and behavior in food systems during their preparation, processing, storage, and consumption. These properties are influenced by the nature and extent of interaction of the proteins with themselves, other components, and water in the food system. The factors that affect the functional properties of food proteins have been classified as intrinsic factors, extrinsic factors, and process treatments or conditions. The food system and the processing dictate most of the extrinsic factors and process treatment/conditions and storage conditions, and hence are amenable to a limited range of maneuverability.

Endopeptidases cleave the peptide linkage between two adjacent amino acid residues in the primary sequence of a protein, yielding two peptides. Factors affecting the enzymatic hydrolysis of proteins include enzyme specificity, extent of protein denaturation, substrate and enzyme concentrations, pH, ionic strength, temperature, and absence or presence of inhibitory substances. The specificity of an enzyme is a key factor, influencing both the number and location of the peptide linkages that are hydrolyzed. Proteolysis can proceed either sequentially, releasing one peptide at a time, or through the formation of intermediates that are further hydrolyzed to smaller peptides as proteolysis progresses, which is often termed the zipper mechanism.

The extent of proteolysis is quantified as the degree of hydrolysis (DH), which refers to the percentage of peptide bonds cleaved. The DH is commonly measured and monitored by the amount of base that is

consumed to maintain the pH during hydrolysis (the pH stat method); by the depression of the freezing point, which indicates the increasing osmolarity (osmometry); or by the increase in solubility in trichloroacetic acid. DH values determined by different methods are often not directly comparable. Good correlation between base consumption and soluble-nitrogen content was established in the case of tryptic hydrolysis of whey protein. The base consumption and osmometry methods are easy to perform, allowing continuous monitoring of the hydrolysis process, whereas the estimation of soluble-nitrogen content using the Kjeldahl method is time-consuming and cannot be used as an on-line process control tool. The effects of enzymatic hydrolysis, using pepsin, Prolase, or Pronase, on some of the functional properties of whey protein were reported as early as 1974.

Infant food formulations are hydrolyzed to a greater extent, and are classified as slightly, moderately, or extensively hydrolyzed, depending on the molecular weight distribution of the resultant hydrolyzate. Extensive hydrolysis is normally used to produce hypoallergenic hydrolyzates, with no peptides greater than 5,000 Da and almost 90% of them approximately 500 Da, whereas hydrolyzates used as protein supplements may undergo less-extensive hydrolysis.

A commonly encountered problem with extensively hydrolyzed proteins is bitterness due to the accumulation of low-molecular-weight peptides containing hydrophobic amino acids. This problem can be solved either by selecting proteases that are not bitter or by adding specific peptidases to debitter the hydrolyzate.

Globular proteins are characterized by specific secondary and tertiary structures involving disulfide linkages and hydrophobic interactions between amino acid residues within the same molecule or between molecules. Three distinct effects accompany enzymatic hydrolysis of proteins:

- A decrease in molecular weight
- An increase in the number of ionizable groups,
- The exposure of hydrophobic groups hitherto concealed

The changes in the functional properties of a protein are a direct result of these effects.

Food-grade proteolytic enzymes have different pH and temperature optima, and they hydrolyze a variety of peptide bonds. Depending on the specificity of the enzyme, environmental conditions, and extent of hydrolysis, a wide variety of peptides are generated. The resultant protein hydrolyzate contains low-molecular-weight peptides as well as higher molecular-weight peptides and unhydrolyzed proteins. Membrane filtration can be used to fractionate the hydrolyzate into a low-molecular-weight permeate and a high-molecular-weight retentate; the functional properties of the two fractions may be vastly different. In the case of whey proteins, permeates have been found to have better foaming and interfacial properties than retentates. However, this additional filtration step decreases yields and increases the production costs, and should therefore be avoided if possible.

Proteases, or proteinases, are the most important class of enzymes from an economic viewpoint. Mucor protease coagulates milk for cheese manufacture. Production of protein hydrolyzates and flavoring materials is discussed below.

Improvement in Functional Properties

There is room for improvement in the functionalities of commonly used food proteins, and physical, chemical, and biological methods of altering proteins have been attempted. Among the most interesting and important is the hydrolysis of proteins by enzymes, which has been covered extensively. Changes in functionality of proteins upon hydrolysis result from the reduced

molecular weight of the peptides, loss or alteration of native structure, and enhanced interaction of peptides with themselves and the environment. The functional properties of milk proteins are well characterized, understood, and used in food applications, but the properties of peptides generated by enzymatic modification are still relatively enigmatic.

Casein, which is used in a variety of foods and whose amino acid composition and sequences are known, has been widely studied for its functional properties. Limited proteolysis improves such surface properties as emulsification and foaming. The foaming property of caseins is important in whipped cream, ice cream, whipped toppings, and mousses.

Controlled enzyme modification of casein and whey proteins by proteolysis generates a total hydrolyzate, comprised of unhydrolyzed substrates, hydrolyzed substrate, and the low-molecular-weight materials. The total hydrolyzate is further fractionated into low-molecular-weight peptides and high-molecular-weight materials. Because the proteolytic reactions are terminated with thermal destruction of the enzyme, sometimes in conjunction with an adjustment of pH, the state of the products in the reaction milieu is usually different from that of the unreacted substrates. A majority of the published research pertains to the total hydrolyzate, while recent research has investigated the nature of the other two fractions from enzyme-modified proteins.

Solubility is considered an essential prerequisite for the manifestation of many functional properties, and enzyme hydrolysis has been directed toward improving solubility of milk protein substrates. Hydrolysis of micellar casein dispersion by alkaline milk proteinase (native to milk) increased the rennet coagulation time after extensive hydrolysis but had no effect on its ethanol stability. Such hydrolysis resulted in a decrease in viscosity of the micellar casein suspension. Further studies showed that the observed increases in soluble nitrogen were due to the production of proteose peptones, rather than the breakdown of the components of the micellar casein. It has been speculated that micellar size might be affected by such hydrolysis. The hydrolyzed micellar casein lowered interfacial tension more than the unhydrolyzed controls, leading researchers to conclude that the peptides generated by such hydrolysis had good surface activity.

Almost all dairy proteins could be improved by treatment with proteases. The increased solubility of enzyme-modified proteins is advantageous in the manufacture of whey-protein-enriched nutritional supplements that are otherwise vulnerable to coagulation and fouling during thermal processing.

Emulsification is facilitated by surfactants, and proteins are considered surface active. Stable coexistence of an oil phase and an aqueous phase in a food system is necessary for good emulsification properties. Dispersion of one phase into another with which it is normally immiscible requires input of mechanical energy, and results in the increase of the interfacial area by several orders of magnitude. Thermodynamic instability leads to the rapid separation of the two phases unless stabilized by surfactants capable of lowering the interfacial tension between the phases. Proteins, by virtue of their amphipathic nature, exhibit good emulsifying properties. Two aspects of the behavior of proteins at the oil-water interface in forming stable emulsions are generally studied. The first is emulsifying capacity, which is a quantitative measure of the amount of oil emulsified by unit weight of the protein. The second is emulsion stability, which measures the ability of the formed emulsion to remain unchanged over time.

Many proteins are surface active and are widely used to control or influence interfacial behavior. The primary and secondary structures of proteins have been thought to be important in the observed emulsifying func-

tionality. However, parts of a protein molecule may consist of amphipathic α-helices and β-sheets with high surface activity, so that a peptide designed to have a maximally stabilized structure should have a higher potential specific surface activity.

As noted previously, hydrolysis of dairy proteins improves emulsification properties. In order to maintain good emulsifying properties the apparent molecular weights of the peptides should not be less than 5,000 Da. In addition, the important role played by enzyme specificity in generating desirable peptides with amphiphilic properties has been demonstrated.

Commercial hydrolyzates of whey proteins varying between 8% and 45% degree of hydrolysis were used to make emulsions with soybean oil. The concentration of hydrolyzates was varied between 0.02% and 5% (wt/wt). The stability of these emulsions was measured by determining the average size of the emulsion droplets and their size distribution both immediately after formation and after storage. The effects of heating on the stability of the emulsions were also determined. As estimated by the particle sizes, the maximum emulsion capacity was observed with a 10% or 20% degree of hydrolysis. Higher degrees of hydrolysis resulted in peptides that were reported to be too small to act as emulsifiers. These researchers reported that at lower than 10% degrees of hydrolysis a lowered solubility diminished the ability of hydrolyzates to emulsify oil. All of the emulsions prepared were unstable to heat at temperatures of 122°C (251.6°F) for 15 minutes, but emulsions prepared with less hydrolysis were stable to heat at 90°C (194°F)for 30 minutes. To a limited extent, mixing different peptides post emulsification could alter emulsion stability.

It has generally been reported in the literature that surface activity is positively correlated with peptide chain length. A minimum chain length of greater that 20 residues has been suggested as necessary for good surface activity. The amino acid composition and sequence of this peptide may also be an important consideration. Such evidence is being provided by studies in which peptides with good surface activity have been isolated and characterized with respect to their chain length and sequence. The environmental conditions of the test also play an important role in the functional properties of the peptides. Additionally, mixtures of peptides may have synergistic or antagonistic effects on surface activity.

Similar to emulsification, foaming requires a protein to be surface active. Dispersion of air in a continuous liquid phase generates a large interfacial area. Stabilization of this air-water interface by surfactants such as proteins or peptides results in the formation of foam. The role and behavior of proteins at the interface is governed by the primary and secondary structures of the peptide chain. A diverse array of products ranging from ice cream to cappuccino gain their identity and structure from the foaming properties of milk proteins. Enzymatic modification of proteins generates peptides with altered foaming properties. In contrast to the emulsification properties of enzyme-modified dairy proteins, relatively little work has been done in the area of foaming properties. It will be interesting to understand the structural and sequential properties necessary to form good foams from these substrates. With advances in genomics, it may be possible to screen for proteases that can specifically hydrolyze the bonds desired, leading to a target production of enzyme–modified ingredients.

Dairy proteins play an important role in gelation, another desirable property in foods. A gel is easier to recognize than define. Qualitative descriptions of gels can help with this recognition. Gels are solids that are able to store the work expended in their deformation and recover to their original shape. Often, gels are soft solids that can be deformed and contain a large proportion of low-molecular-weight liquid. Thus, gels

exhibit both the elastic and viscous properties described by the term viscoelasticity. The gelation process can immobilize a large volume of liquid.

A classic example of gelation of milk occurs in the cheese-making process wherein the enzyme chymosin causes the formation of a soft gel. This gelation occurs due to the hydrolysis one specific peptide bond (Phe105-Met106) in κ-casein by chymosin. The scission of the glycomacropeptide causes the casein micelle to become sensitive to ionic calcium in the environment, leading to coagulation or gel formation.

Any alteration in the native conformation of a protein has the potential to induce gelation. Thus, enzymes modify the conformation of proteins and the altered conformation increases the propensity of the protein to form gels under appropriate conditions. Sometimes other enzymes, e.g., transglutaminase, are employed to increase the strength of the formed gels. The exact nature of the peptides that result in gelation is not easy to understand. The processing pre-treatments accorded to proteins pre- or post-modification confound the understanding of the gelation process. In general, enzymatic modification results in a conformation of the protein that can form aggregates, which have a propensity to form gels under a variety of conditions. Altering the environment by changing pH, ionic strength, and type of ions, or by physical treatments such as heat causes the aggregates to form gels. Structural information in this context is of limited use in delineating the mechanism of gelation.

Flavor Production Resulting from Hydrolysis

Commonly used hydrolyzed proteins for flavorings are derived from plant materials, and milk proteins are not an economically viable source for manufacturing such flavorings. However, enzymes are used to manufacture cheese flavors. Lipids and proteins are the two main substrates for the manufacture of cheese flavors. The use of enzyme-modified cheese (EMC)and lipid flavors is discussed later in this chapter.

A variety of proteases is commercially available. The operational characteristics (pH, temperature of stability, specificity, etc.) and amount and type of products vary, and the variations result in different flavor profiles in EMC. Twenty-three commercial microbial proteinase preparations derived from various Bacillus or Aspergillus spp. or from *Rhizomucor niveus* were assessed for proteolytic activity on azocasein at pH 5.5 or 7.0 or specificity on sodium caseinate at pH 5.5. They were semi-quantitatively assessed for esterase, lipase, trypsin, chymotrypsin, general aminopeptidase, phosphatase, and glycosidase activities using the API-ZYM system. Selected preparations were further assayed for peptidase, esterase, and lipase activities at pH 7.0. The proteolytic activity of the Bacillus preparations was greater at pH 7.0, while that of the Aspergillus and Rhizomucor preparations was greater at pH 5.5. All of the Bacillus preparations contained one of two main proteolytic activities, thought to be either bacillolysin or subtilisin. Most of the Aspergillus preparations contained the same proteinase, thought to be aspergillopepsin I, but two preparations appeared to contain a different unidentified proteinase. The proteolytic specificity of the Rhizomucor preparation was different from that of the Bacillus or Aspergillus preparations; this difference is thought to be due to an enzyme called rhizopuspepsin.

According to the results of the API-ZYM system, all preparations contained enzyme activities in addition to their main proteolytic activity, with the Aspergillus and Rhizomucor preparations containing the highest levels and widest range of activities. Generally, preparations derived from Aspergillus contained the highest level of general, proline, and endopeptidase activities, with the Bacillus preparations conspicuous by the

absence of general and proline-specific peptidase activities. The *Rhizomucor niveus* preparation contained little or no general or endopeptidase activity. Esterase activity was found in all of the preparations evaluated, with only two Aspergillus preparations containing lipase activity.

Lipid Modification

Enzymatic modification of lipids is facilitated by lipases and esterases. Lipases hydrolyze triglycerides to free fatty acids and mono- and di-glycerides. Lipases require an insoluble substrate to be present at an interface; with triglycerides the interface is created by emulsifying the substrate in an aqueous medium. A potential hindrance to lipolysis is the generation of surface-active mono- and di-glycerides that block the interface. If such surfactants are not controlled, the rate of lipolysis continues to decrease. One method of controlling the release of surfactants is to include divalent cations, e.g. Ca^{2+} , which can form insoluble soaps.

As noted above, plants, animals, and microorganisms produce lipases. Plant lipases derived from castor bean or wheat germ are not commercially viable sources of enzyme. Animal lipases are mainly obtained from porcine or bovine pancreas. Other enzymes derived from animals are caprine (kid and goat) pre-gastric lipases and esterases.

Microbial lipases are extracellular enzymes and in some instances inducible. The synthesis of the enzyme is under feedback control of mono- and di-glycerides and glycerol concentrations in the growth medium. Some microbial lipases are glycoproteins with carbohydrate moieties facilitating the secretory process. Most microbial lipases have pH optima in the alkaline range (pH 8 to 9) and altering composition of the growth medium by salts and emulsifiers can shift the optima to an acidic range. Most industrial microbial lipases are active between 30°C to 40°C (86°F to 104°F). On the other hand, lipolytic rancidity has been observed in foods stored at -29°C (-20°F). Some lipases, referred to as non-specific, hydrolyze fatty acids at any position in the triglyceride. Non-specific lipases break down triglycerides to free fatty acids and glycerol. Other lipases, referred to as specific, hydrolyze fatty acids esterified at one or three position of glycerol. The products of specific lipase reactions are free fatty acids and di- and mono-glycerides. Lipases, in addition to positional specificity, also exhibit specificity towards the type of fatty acid. Pancreatic lipase is specific for shorter chain fatty acids esterified to glycerol. Milk fat contains short chain fatty acids.

Pre-gastric esterases are useful in generating specific flavors reminiscent of Italian cheeses. Esterases act on soluble substrates as opposed to lipases, which require insoluble substrates. Pre-gastric esterases are also known as pre-lingual lipases—a misnomer. The main sources of esterases are kid, calf, and lamb. These enzymes, used in traditional Italian piquant cheeses, are not acceptable to vegetarians and are very expensive. As judged by enzyme activity on tributyrin substrate, pre-gastric esterases are 1,000 times more expensive than pancreatic lipase. The cost disadvantage of pre-gastric esterases coupled with animal virus issues have led to the use of microbial esterases. Besides being less expensive, microbial esterases are protease free and useful for vegetarian products.

The free fatty acids that are generated are converted to flavor compounds by reactions such as thiolation, oxidation, and esterification, yielding compounds such as thiolesters, γ- or δ- lactones, ethyl esters, and alken-2-ols. Thiol ester formation involves sulphur-containing amino acids such as methionine and cysteine, whereas interaction of free fatty acids with tryptophan results in indole, and tyrosine forms phenol. The amino acid leucine reacts with α-ketoglutarate to form α-isoketocaproate. Phenylalanine catabolism

forms phenyl lactate, benzaldehyde, phenylacetate, and phenylethanol. These are a few examples of flavor-impact compounds present in cheese; they reflect the chemical complexity of cheese flavor.

Ingredients Involving Proteolysis and Lipolysis

Proteolysis

Proteolysis of casein and whey proteins leads to products called casein hydrolyzate and whey protein hydrolyzate. For each type of protein, various degrees of hydrolysis can be achieved. Casein hydrolyzates are often bitter due to the preponderance of hydrophobic amino acids. Whey protein hydrolyzates are non-bitter but astringent.

Milk and whey peptides are latent until released and activated, e.g., from digestion or milk fermentation. Once activated, these peptides have many bioactive functions, all of which relate to general health conditions or reducing the risk of certain chronic diseases. Some examples of the physiological effects of peptides are given below. These proteins are subject to considerable research worldwide. The best data so far indicate that ACE-inhibitory peptides limit the formation of angiotensin II, a potent chemical that causes the muscles surrounding the blood vessels to contract and thereby increasing blood pressure.

Recent studies have indicated that dairy peptides are opioid peptides and may play an active role in the nervous system. Once absorbed into blood, these peptides can travel to the brain and other organs and cause pharmacological properties similar to opium and morphine. This may be why newborn infants generally become calm and sleepy after drinking milk. It also has been suggested that these peptides may alleviate allergic reactions in humans and enhance mucosal immunity in the gastrointestinal tract; thus, peptides may regulate the development of the immune system in newborn infants. Furthermore, immunopeptides formed during milk fermentation may contribute to the anti-tumor effect of fermented milk.

Casein Hydrolyzates

Hydrolyzed casein is a rich source of peptides, many of which have physiological effects. The properties of α-casein hydrolysates differ, depending upon the enzymes used to digest them. Trypsin hydrolysis of α-casein promotes antibody formation and phagocytosis (the engulfing of the cell wall of external cell material, such as bacteria) and reduces the severity of infections. Residues 90 to 96 (Arg-Tyr-Leu-Gly-Tyr-Leu-Glu) and 90 to 95 (Arg-Tyr-Leu-Gly-Tyr-Leu) of α-casein have been shown to have opioid properties, increasing the body's natural killer cells' (lymphocytes) responses and boosting the number of white blood cells.

Beta-casein is the source of numerous bioactive peptides. Residues 63 to 68 (Pro-Gly-Pro-Ile-Pr-Asn) and 191 to 193 (Leu-Leu-Tyr) of β-casein promote antibody formation and phagocytosis. Other peptides derived from β-casein have anti-hypertensive activity, promote mineral absorption, or have an opiate-like effect (β-casomorphins). Furthermore, these peptides are considered to be immune suppressive when taken orally. Because immunosuppresion prevents the body from rejecting organ and bone marrow transplants, or is used to treat auto-immune diseases such as Crohn's disease, work within this field could open up a new market for β-casein peptides.

Similar to the other casein peptides, the digestion of κ-casein results in several peptides, which adjust or change the immunomodulatory responses of the body. The κ-casein peptide Phe-Phe-Ser-Asp-Lys promotes antibody formation and phagocytosis, while the κ-casein peptide Tyr-Gly increases the generation of lymphocytes in the human body.

Glycomacropeptide (GMP) is a carbohydrate-rich peptide present in liquid whey. It is derived from the splitting of κ-casein by chymosin, or rennet, during cheese or casein production. The peptide residues of this process are soluble and therefore become part of the whey. There are two different variants of GMP, A and variant B, which differ in two amino acids. Different abbreviations are often used for GMP, such as CGMP or CMP, but these all refer to the same molecule.

GMP is rich in sialic acid. Studies indicate that sialic acid contained in GMP promotes the growth of bifidobacteria and is involved in the brain development of newborns. It is also believed to play an anti-infective role in the small intestine. The peptides in GMP have a high content of branched chain amino acids (BCAA) and contain no aromatic amino acids, which makes them ideal ingredients in nutritional formulations for people suffering from hepatic diseases. GMP is also an ideal nitrogen source for people suffering from phenyl-ketonuria (PKU), due to the lack of phenylalanine. A person suffering from PKU is not able to metabolize phenylalanine, which causes it to accumulate and damage the central nervous system and possibly the brain. Furthermore, studies over the past thirty years have shown that GMP may influence satiety in humans, inducing the secretion of cholecystokinin (CKK), a group of neuropeptides known to regulate short-term control of food intake. However, this effect seems to depend on the actual structure and composition of GMP. GMP has also demonstrated a protective effect on teeth by inhibiting cariogenic bacteria.

Caseinphosphopeptides (CPP) are a type of phosphorylated casein-derived peptides. Interest in this ingredient is increasing due to its purported ability to bind and solubilize minerals such as calcium, zinc, iron, and magnesium. CPP forms a stable complex with calcium phosphate, which hinders calcium phosphate precipitation and thus increases bioavailability of calcium. CPP also maintains a state of supersaturation with respect to tooth enamel, depressing demineralization and enhancing remineralization and preventing tooth decay. CPP is thought to add value to a number of different oral hygiene products such as toothpaste, mouthwash, and chewing gum. CPP can be produced industrially from sodium caseinate using enzymes. CPP can dissolve in a wide range of pH levels, is heat stable, and can be processed at high temperatures. CPP applications include health foods, dairy products, soy products and carbonated beverages such as beer (CPP makes foam smaller and longer lasting). Some commercially available casein-derived manufacturers and products are listed in Table 12.1 and currently marketed products are listed in Table 12.2.

Table 12.1. Hydrolyzed casein (milk protein hydrolyzate) manufacturers.

Producer	MPH product name
American Casein Co.	HLA-198
Arla Food Ingredients	LACPRODAN and PEPTIGEN
Armor Proténes	Vitalarmor 950
Ingredia	NA
Lactalis	NA
Kerry Ingredients	NA
Morinaqa	NA
Fonterra	NA
FrieslandCampina	NA
Glanbia	BarPro

Whey-protein-derived Peptides

Whey represents a rich and heterogeneous mixture of secreted proteins with wide ranging nutritional, biological, and food functional attributes. The main constituents are β-lactoglobulin (β-lg) and α-lactalbumin (α-la), two small globular proteins that account for approximately 70% to 80% of total whey protein. Historically, whey has either been considered a waste product and disposed of in the most cost-effective manner, or processed into relatively low value commodities

Table 12.2. Currently marketed dairy products containing functional peptides.

Sample	Peptide	Product	Function	Producer
Calpis	VPP/IPP	Sour milk	Hypotensive	Calpis Co (JP)
Evolus	VPP/IPP	Fermented milk	Hypotensive	Valio (FI)
Capolac	CPP	Ingredient	Mineral absorption	AFI (DK)
PeptoPro	NA	Ingredient	Recovery	DSM Foods (NL)
tensVida	IPP	Ingredient	Hypotensive	DSM Foods (NL)
Cysteine Peptide	Milk-protein-derived peptide	Ingredient	Raise energy and sleep	FrieslandCampina (NL)
C12	FFVAPFPEVFGK	Ingredient	Hypotensive	FrieslandCampina (NL)
PRODIET F200/ Lactium	TLGTLGGLLA	Ingredient	Anxiety reduction	Ingredia (FR)
NOP-47	NA	Ingredient	Vascular function	Glanbia Nutritionals

such as whey powder and various grades of whey protein concentrate (WPC) and isolate (WPI).

Isolation of whey proteins as spray-dried whey powder and, in more limited quantities, as whey protein concentrate/isolate, has realized only a small portion of the commercial potential of these proteins. Indeed, whey protein concentrate, once heralded as a value-added outlet for whey solids, is now considered a commodity item. In addition, whey-protein-based products have an unfortunate record of inconsistent and unreliable performance in food systems. Thus, expanded use of whey proteins relies on exploitation of individual whey proteins and their derivatives as products with increased nutritional, functional, and/or biological value and increased commercial value to the dairy industry. The emergence of new technologies and methods provides fresh insight into the bioactivity of these proteins and produces new and sometimes surprising results.

β-lg is the predominant protein in cheese whey. Various peptides derived from proteolytic digestion of β-lg have been shown to have an inhibitory activity against angiotensin-converting enzyme (ACE), which plays a major role in the regulation of blood pressure and thereby hypertension. It has been shown that unhydrolyzed β-lg had very poor ACE inhibitory activity, but that digests of the protein, generated using pepsin, trypsin, chymotrypsin, or other commercially available proteases, resulted in high ACE inhibition indices (i.e. 73% to 90%). Furthermore, the active peptides were usually short (3 to 8 amino acids) and could be enriched from a mixture of protein and other peptides using ultrafiltration with low-molecular-mass cut-off membranes.

A tryptic peptide of β-lg (f142 to 148) was further characterized following reversed-phase chromatographic isolation and shown to have an ACE IC50 value of 42.6 nM. Similarly, several researchers have demonstrated that a number of β-lg-derived peptides have impressive ACE inhibitory activity using a variety of *in vitro* assay techniques. In a study in which whey proteins were treated with different lactic acid starters and digestive enzymes, it was reported that two peptides from β-lg (f9 to 14 and f15 to 20), following hydrolysis with trypsin or pepsin and characterization by amino acid and MS-analysis, had ACE inhibitory activity (Meisel, 1998). Four novel ACE inhibitory peptides have been reported from caprine β-lg following hydrolytic treatment with thermolysin and purification. It has been demonstrated that a tetrapeptide isolated from β-lg (f142 to 145; Ala-Leu-Pro-Met), termed β-lactosin B, had significant anti-hypertensive activity when administered orally to spontaneously hypertensive rats (SHR) and therefore has

potential as a natural antihypertensive agent for inclusion in foods.

Peptide fragments of β-lg, generated through the action of alcalase, pepsin, or trypsin, have been shown to be bacteriostatic against *E. coli* and against pathogenic strains of *E. coli, Bacillus subtilis*, and *Staphylococcus aureus* (Meisel, 1998).

Alphalactalbumin (α-la) is the next most predominant protein in whey. The peptide with the amino acids sequence Tyr-Gly-Leu-Thr (f50 to 53), released from α-la by pepsin treatment, was shown to inhibit ACE; the accuracy of the last amino acid (Thr) is uncertain. This peptide has been termed α-lactophorin. Interestingly, proteolytic fragments of this peptide, i.e., the dipeptides Tyr-Gly (f18 to 19 and f50 to 51) and Leu-Phe (f52 to 53), were also observed to have an inhibitory effect. Trypsin treatment of α-la has been shown to release two antibacterial peptides, Glu-Gln-Leu-Thr-Lys (f1 to 5) and Gly-Tyr-Gly-Gly-Val-Ser-Leu-Pro-Glu-Trp-Val-Cys-Thr-Thr-Phe (f17 to 31) disulphide-bonded to Ala-Leu-Cys-Ser-Glu-Lys (f109 to 114). Treatment using another intestinal enzyme, chymotrypsin, resulted in one antibacterial peptide, namely, Cys-Lys-Asp-Asp-Gln-Asn-Pro-His-Ile-Ser-Cys-Asp-Lys-Phe (f61 to 68) disulfide bound to f75 to 80 (Chatterton et al., 2006). These peptides were mostly active against Gram-positive bacteria; however, weaker effects were observed with Gram-negative bacteria. Although pepsin did not release any antibacterial peptides in one study, a different study indicated that both pepsin and trypsin released peptides from α-la, which inhibited the growth of *E. coli* JM103. The peptide concentration was 25 mgmL⁻1, whereas unhydrolyzed α-la did not inhibit the growth at a concentration of 100 mgmL⁻1. Peptides released from whey proteins and their bioactivities are shown in Table 12.3 and commercially available whey peptide products are shown in Table 12.4.

Ingredients Derived from Lipolysis

A majority of the flavor compounds are derived from lipids or are a result of interactions of lipolysis and proteolysis products. Most flavor-impact compounds are also lipid soluble. Recent trends in healthy foods have created markets for low- and nonfat products in the United States. Nonfat products do not have enough lipids to act as effective flavor carriers. The time of onset of flavor release and the duration of flavor perception are

Table 12.3. Peptides derived from whey proteins and their bioactivity.

Precursor protein	Fragment	Peptide sequence	Name	Function
α-lactalbumin	50–53	Tyr-Gly-Leu-Phe	α-lactorphin	Opioid agonist, ACE inhibition
β-lactoglobulin	102–105	Tyr-Leu-Leu-Phe	β-lactorphin	Non-opioid stimulatory effect on ileum, ACE inhibition
	142–148	Ala-Leu-Pro-Met-His-Ile-Arg		ACE inhibition
	146–149	His-Ile-Arg-Leu	β-lactotensin	Ileum contraction
Bovine serum albumin	399–404	Tyr-Gly-Phe-Gln-Asn-Ala	Serorphin	Opioid
	208–216	Ala-Leu-Lys-Ala-Trp-Ser-Val-Ala-Arg	Albutensin A	Ileum contraction, ACE inhibition
Lactoferrin	17–41	Lys-Cys-Arg-Arg-Trp-Glu-Trp-Arg-Met-Lys-Lys-Leu-Gly-Ala-Pro-Ser-Ile-Pro-Ser-Ile-Thr-Cys-Val-Arg-Arg-Ala-Phe	Lactoferricin	Antimicrobial

Table 12.4. Commercial whey protein hydrolyzate (WPH) ingredients in the marketplace.

Producer	WPH product name
American Casein Co.	HCA-411
Arla Food Ingredients	LACPRODAN and PEPTIGEN
Armor Proténes	Vitalarmor 855LB
Carbery	Optipep, Optipep DH5A
Davisco	NA
Fonterra	NA
FrieslandCampina	NA
Glanbia	Barflex
Hilmar Ingredients	Hilmar 8360 to Hilmar 8390
Ingredia	NA
Lactalis	NA
Kerry Ingredients	NA
Morinaqa	NA
Murray Goulburn	NA
Milk Specialties Global	9003, 9004, 9005, 8010, 8020, 9010, 9015, 9020
Protient	3020, 4003, 4020
Tatua	NA

related to the amount of fat in the product. The partition coefficients of flavor compounds in an oil-in-water system influence vapor pressure and, therefore, volatility of these molecules. In a fat-free system only a single aqueous phase exists, and release and duration of flavor compounds is drastically different than in oil-in-water systems.

Cost reduction of food formulations is important, especially in cost-sensitive (economic) products. Costs can be lowered by careful control of the types and quantities of ingredients used in formulations. Milk fat and milk protein are expensive ingredients. Therefore, alternative, less expensive ingredients are desirable in the manufacture of certain products (e.g., cream in desserts, cheese in dips, or blue cheese in salad dressings). Local availability and cost of dairy ingredients also factor in the selection of ingredients in food formulations.

Enzyme-modified Butterfat

Many different processes for modifying butterfat are available to flavor manufacturers. Milk-fat emulsions are modified to make enzyme-modified cream and enzyme-modified butter oil. Bread formulations have been evaluated for the effects of enzyme-modified butter oil on the attributes of the baked product. Commercial shortening served as the control and another control used 3% butter oil, while the experimental samples contained 2% butter oil plus 1% enzyme-modified butter oil, 1% butter oil plus 1% enzyme-modified butter oil, and 2% commercial shortening plus 1% enzyme-modified butter oil (Arnold et al. 1975). The breads were judged by a trained sensory panel for flavor, color, softness, appearance, and internal structure. The experimental breads were judged to be slightly softer, and samples with enzyme-modified butter oil were superior in flavor to the two controls. After 24 hours of storage, the control bread was stale and the experimental bread was fresh. Enzyme-modified butter oil can be substituted for 35% to 40% of the shortening. Butter oil modified with enzymes derived from *Achromobacter lipolyticum*, *Penicillium roquefortii,* or *Geotrichum candidum* is not recommended for baked goods because they produce musty and soapy flavored bread.

Symrise Company markets Dariteen L-11 and L-40 lipolyzed creams; Dariteen L-22, a lipolyzed cultured cream product; and Dariteen L-60 and L-95, natural dairy flavors with a strong butter flavor. Lipolyzed cream products are natural dairy flavors produced by treating fresh cream with lipase. Hydrolysis of butterfat in cream liberates the flavorful free fatty acids butyric, caproic, caprylic, and capric. To control flavor development in the final product, the lipolyzed cream is heat treated to denature lipases. Lipolyzed cultured cream products are inoculated with *Lactobacillus delbrueckii* subsp. *bulgaricus* to develop acidity in cream prior to lipolysis. After lipolysis, a heat treatment is used to destroy both the enzyme and the culture bacteria.

Pure butter oil is also lipolyzed, and the modified product is solid at room tempera-

ture. Lipolyzed cream and cultured cream enhance dairy flavors in candies, cheesecakes, sauces, dips, salad dressings, sweet doughs, soups, and baked goods. For subtle flavor, the modified creams are used at the 0.05% to 0.1% level, and for a more pronounced effect, levels of 0.1% to 0.5% are customary. Partially lipolyzed butter oil is useful in flavoring oils, fats, cereals, snacks, and baked goods. For example, the oil used to cover popped corn results in buttered popcorn. In this application, the typical use level of the modified butter oil is 0.05% to 1% in the finished food; these flavors are heat stable and are suitable where high temperatures are used during preparation or processing of the food.

Caramel candy manufacture consists of mixing dry cream with water followed by the addition of corn syrup, salt, and vegetable oil. This mixture is cooked to 110°C (230°F) and evaporated milk is added under continuous agitation. Vanilla is then added and the candy cooled, cut, and wrapped. Dariteen L-22 is used in caramel manufacture and a typical formulation is shown in Table 12.5. In another candy formulation, for butterscotch (Table 12.6), Dariteen L-95 is used. Butterscotch hard candy is made by combining sugar, corn syrup, salt, water and vegetable oil and cooking the mixture to 136°C (277°F) prior to adding Dariteen L-95, coloring the mixture, and raising the temperature to 138°C (280°F). The candy is cooled, cut, and wrapped.

Table 12.5. Formulation for caramel candy using lipolyzed cultured cream.

Ingredient	Quantity (wt.%)
Corn syrup (42 DE)	30.92
Evaporated milk	31.32
Sugar	19.32
Water	10.83
Dry cream (72% butterfat)	4.64
Hydrogenated vegetable oil	1.55
Dariteen L-22	1.00
Salt	0.20
Vanilla extract	0.20
Lecithin	0.20
TOTAL	100

Table 12.6. Formulation for butterscotch hard candy using lipolyzed butter oil.

Ingredient	Quantity (wt.%)
Sugar	51.88
Corn syrup (43 DE)	31.12
Water	14.52
Hydrogenated vegetable oil	1.03
Dairyteen L-95	1.00
Salt	0.45
Color	As desired
Total	100

Cheese Flavors

Enzyme-modified cheese flavors are used in a variety of foods. Proteases along with esterases are used in a ratio of 1 : 2 to 1 : 3 to treat cheese curds over a certain period at 10°C to 25°C (50°F to 70°F). As depicted in Figure 12.1, the use of discreet proteases helps in generating desirable flavors.

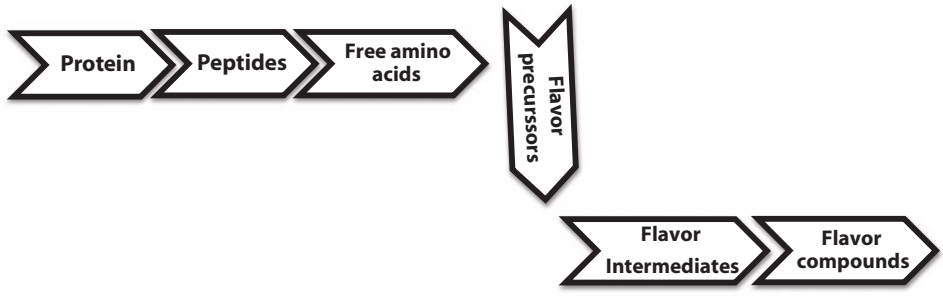

Figure 12.1. Contribution of proteolysis to flavor compounds.

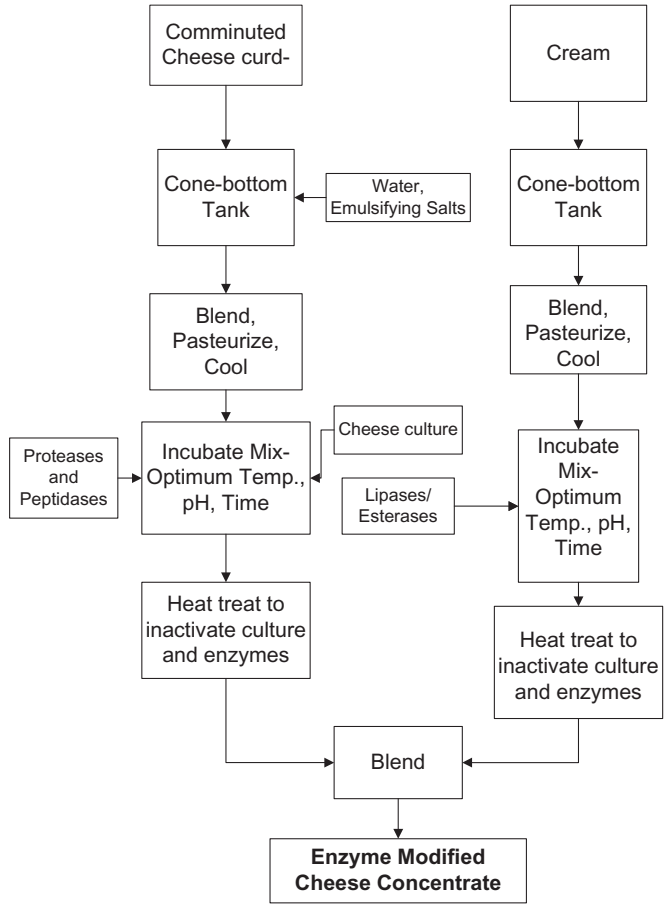

Figure 12.2. Principles of enzyme-modified cheese production. Adapted from Wilkinson and Kilcawley (2003).

In some cases, cheese cultures are also added. The resulting material has five- to 15-fold strength of a regular cheese flavor. The exact reaction conditions and particulars are closely held trade secrets. Figure 12.2 shows the principles involved in EMC production. After incubation to maximize the cheese flavor, the blend is heat treated to inactivate the enzymes and cultures and spray dried. EMC paste is also available.

A variety of flavor profiles can be developed of varying compositions and product forms. Typical applications include processed cheese, cheese spreads, cheese dips, cheese analogs, and cheese sauces. The flavor quality of these products can be significantly enhanced by the use of EMC without increasing the quantity of cheese in a product.

EMC also can replace cheese in food formulations; the levels used depend on the amount of cheese being replaced and the flavor intensity of the EMC. For example, if a current formulation has 50% cheese and a 50% substitution is desired with an EMC that is 20 times more potent than the cheese flavor, only 1.25% of the EMC is required to reach the substitution target without affecting flavor intensity. However, this reduces the weight of the formulation, and other ingredients such as starch and oil may have

to be added to restore the weight of the original formulation. Various EMCs are available to boost or replace cheddar, Swiss, blue, Romano, and other cheese varieties.

Generally, in the manufacture of enzyme-modified dairy ingredients, the substrate is prepared and then the enzyme solution is prepared and standardized. The enzyme solution is added to the substrate and the mixture is homogenized or thoroughly mixed. The mixture of enzyme and substrate is incubated at an appropriate temperature to achieve the desired conversion of the substrate to the desired product. This is followed by enzyme inactivation, spray drying, and packaging.

Differing amounts of products are yielded when using the same substrata but different lipolytic enzymes. The addition of pre-gastric esterase in the manufacture of the Italian Romano and provolone cheeses results in the much desired piquant flavors. Lamb gastric esterase in combination with lamb pre-gastric esterase results in provolone-like flavor. *Mucor miehei* esterase is used to develop flavor in Romano and fontina cheeses. Inclusion of pre-gastric esterase and fungal esterase (on an equal activity basis) results in good fontina flavor but five times more fungal esterase than the pre-gastric kid esterase that is required to develop good Romano cheese flavor.

Romano cheese flavor from Symrise Company contains Romano cheese, water, salt, and potassium sorbate. The product is available in 25-kg (approximately 50 lbs) polyethylene-lined pails. Romano cheese is ground through a 0.63-cm (1/4-inch) plate and mixed with warm water at 60°C to 70°C (140°F to 158°F) to obtain a lump-free slurry. The remaining ingredients are added and the temperature is raised to 82°C (180°F) and held for 15 minutes prior to filling in containers, which are then thermally processed. A formulation for a tomato sauce with Romano cheese is given in Table 12.7.

Table 12.7. Tomato sauce with Romano cheese made with and without Dariteen-310.

Ingredients	Content (wt.%)	
	Without Dariteen	With Dariteen
Tomato puree	44.02	44.02
Water	26.83	28.83
Tomato paste	17.77	17.77
Romano cheese	6.00	3.00
Dariteen-310	0	1.00
Salt	0.62	0.62
Dried onion	0.53	0.53
Sugar	0.49	0.49
Oregano	0.18	0.18
Garlic powder	0.01	0.01
Total	100	100

Blue cheese is another popular cheese flavor; it involves four enzymatic steps. First, fatty acids are liberated from milk fat by lipases. The released fatty acids are oxidized to β-keto acids, which undergo decarboxylation to generate methyl ketones, which in turn are reduced to yield secondary alcohol. Blue cheese flavors can be produced by submerged mycellial fermentation involving sterile milk or milk fat. The flavors are used in salad dressings, dips, products that can be extended, and baked snack foods. The levels used in food products vary, depending on the process used to obtain the flavor. For example, a flavor obtained by continuous fermentation and containing 24 times the concentration of C 5, 7, 9, or 11 methyl ketones than in blue cheese can replace 100% blue cheese in a chip dip.

Compared to Italian and blue cheese, cheddar, Swiss and Dutch cheeses undergo low levels of lipolysis. Addition of rennet paste, pre-gastric esterase, or gastric lipase improves the flavor of cheddar cheese and several patents have been granted for such an application. Enzyme-modified cheddar cheese flavor comes in various intensities, such as mild, sharp, and extra sharp. Symrise company has a five-times paste and an eight-times cheese powder. All of these flavors they are blended with other natural flavors (WONF). Typical applications of enzyme-modified cheddar cheese include cheese

Table 12.8. Ingredient formulation for cheddar cheese soup with and without Dariteen enzyme-modified flavor.

Ingredient	Content (wt.%)	
	Without Dariteen	With Dariteen
Water	52.59	53.83
Sharp cheddar cheese	20.00	16.00
Nonfat dry milk	12.00	1.23
Whey powder	5.00	4.00
Flour	4.00	4.40
Oil	4.00	5.28
Dariteen-245 Cheddar flavor	0	0.75
Dariteen L-22 lipolyzed cream flavor	0.60	0.60
Corn starch	0.50	0.50
Salt	0.40	0.50
Tureen AYP 65 yeast extract	0.20	0.20
Oleoresin capsicum	0.10	0.10
Oleoresin paprika	0.01	0.01
Total	100	100

Table 12.9. Formulation for a frozen Swiss cheese sauce with and without Dariteen-410 Swiss cheese flavor.

Ingredients	Quantity (wt. %)	
	Without Dariteen	With Dariteen
Water	71.7	74.4
Swiss cheese (aged more than 60 days)	15.0	11.3
Nonfat dry milk	6.0	6.0
Buttermilk solids	3.0	3.0
Oil	2.0	2.0
Starch	1.5	1.5
Dariteen-410 Swiss cheese flavor	0	1.0
Salt	0.4	0.4
Tureen AYP 65 yeast extract	0.3	0.3
Xanthan gum	0.1	0.1
Annatto extract	As desired	As desired
Total	100	100

analogs, spaghetti sauces, and cheese sauces. Typical application of Dariteen-245 (a 20-times sharp cheddar flavor) in a soup formulation is provided (Table 12.8).

The process steps for the manufacture of this product are: First grind the cheese through a 0.31 cm (0.25") plate. Combine non fat dry milk, whey, flour, corn starch, salt, monosodium glutamate and Tureen AYP 65 yeast extract with water and oil. Add the grated cheese, Dariteen 245 and Dariteen L-22, paprika and capsicum oleoresins, mix and heat to 88°C (190°F) and hold for 10 minutes., fill cans and thermally process the containers. This is a condensed soup and can be hydrated with an equal part of water prior to consumption.

Swiss cheese flavor for use in fondues, quiches, crackers, and sauces also is commercially available. A typical formulation (Table 12.9) is processed by grinding cheese through a 0.31-cm (0.25-inch) plate. Nonfat dry milk, buttermilk solids, starch, salt, Tween AYP 65, and xanthan gum are mixed thoroughly in a kettle. This mixture is combined with water and grated Swiss cheese and oil are added, along with Dariteen 410 and annatto extract. The mixture is heated to 88°C (190°F) for five minutes and the product is packaged and frozen.

Strongly flavored cheese is produced by adding protease and lipase mixtures to cheese curd and then curing at 10°C to 25°C (50°F to 77°F) 1 to 2 months. The ratio of esterase to lipase activity should be 2 or 3 : 1. Over treatment results in the methyl ketones 2-heptanone and 2-nonanone. Concentrated cheese flavorings are produced by the rapid modification of slurries of milk solids or casein, various fats, and emulsifiers.

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Chapter 13

Fermented Dairy Ingredients

Junus Salampessy and Kasipathy Kailasapathy

Introduction

> "When people have learnt how to cultivate a suitable flora in the intestine of children as soon as they are weaned from the breast, the normal life may extend to twice my 70 years."

That rather prophetic statement by Élie Metchnikoff (1921) has arguably never been achieved because there is no report to date that humans can normally live up to 140 years. However, the statement emphasizes maintaining balanced intestinal flora to have a healthy life. Over the last few decades the market for fermented food products, especially fermented dairy products such as yogurt, has increased significantly along with the steadily increasing knowledge of the health benefits of fermented dairy products. The health benefits of fermented dairy products, however, are not new. Various studies have been carried out for many years to investigate health-related activities of fermented milk products. Fermented dairy products have been human staples for centuries; they come from different parts of the world and in different forms, flavors, and textures. This chapter discusses various fermented milk products, especially mesophilic-acid-coagulated and thermophilic products, and their use as dairy ingredients.

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Microflora of Milk Fermentation

The type and characteristics of microorganisms used as starter cultures for milk fermentation are two of the most important factors that determine the overall quality of the fermented products (Bouzar et al. 1997). A proper selection and composition of starter culture can improve the product flavor, aroma, stability, and texture. Therefore, it is of great importance to understand the characteristics of the starter bacteria to obtain desirable, quality products. The microorganisms commonly used in various milk fermentations are listed in Table 13.1.

The microorganisms used in milk fermentation can be categorized into three groups: mesophilic, thermophilic, and artisanal. The optimum temperatures for the first two groups are approximately 26°C (79°F) and 42°C (108°F), respectively, and they consist of different species of bacteria. The artisanal is an undefined bacterial culture that is produced daily in cheese plants and contains various numbers and types of lactic acid bacteria (LAB) (Cogan 1995). Furthermore, the starter bacterial culture can be classified into acid- and flavor-producing bacteria. The acid-producing bacteria mainly convert milk sugar, or lactose, into lactic acid and, to a lesser extent, acetic acid, through a homofermentative pathway. This group includes *Str. Thermophilus*; *Lb. delbrueckii* subsp. *Bulgaricus*; *Lb. helveticus*; and some *Lactococcus*, *Leuconostoc*, and *Bifidobacterium* species (Monnet et al. 1995).

Table 13.1. Microflora commonly used for selected milk fermentation.[a]

Product category	Product names	Raw material	Starter culture	Citrate metabolism[b,e]	Isomer of lactate[b,f]
Mesophilic fermentation (Soft unripened cheese)	Cottage cheese	Skim milk	*Lc. lactis* subsp. *cremoris*	–	L
			Lc. lactis subsp. *lactis*	±	L
			Leuc. mesenteroides subsp. *cremoris*	+	D
	Quark	Skim milk	*Lc. lactis* subsp. *cremoris*	–	L
			Lc. lactis subsp. *lactis*	±	L
			S. diacetylactis[c]	+	D
	Cream cheese	Cream	*Lc. lactis* subsp. *cremoris*	–	L
			Lc. lactis subsp. *lactis*	±	L
			Leuc. mesenteroides subsp. *cremoris*	+	D
	Cultured buttermilk	Skim milk	*Lc. lactis* subsp. *cremoris*	–	L
			Lc. lactis subsp. *lactis*	±	L
			Lc. lactis subsp. *lactis* biovar. *Diacetylactis*[c]	+	D
	Sour cream	Cream	*Lc. lactis* subsp. *cremoris*	–	L
			Lc. lactis subsp. *lactis*	±	L
			Leuc. mesenteroides subsp. *cremoris*	+	D
			Lc. lactis subsp. *lactis* biovar. *Diacetylactis*[d]	+	D
Thermophilic fermentation	Yogurt	Milk	*S. thermophilus*	–	L
			Lb. delbrueckii subsp. *bulgaricus*	–	D

[a]Farkye (2007) unless stated otherwise
[b]Cogan (1995)
[c]Walstra et al. (2006)
[d]Kosikowski and Mistry (1997)
[e]Symbols +, –, and ± indicate the ability, inability, or both of the microflora to produce citric acid
[f]Indicates the ability of the microflora to produce different isomers of lactate

The flavor-producing bacteria convert citrate in milk into acetate, diacetyl, acetoin, 2,3-butanediol, and carbon dioxide, which are responsible for the flavor of fermented milk products. Diacetyl is an important flavor component of various fermented milk products such as cultured buttermilk, sour cream, fromage frais, and quark. The metabolism of citrate is an important property of some mesophilic, but not thermophilic, bacteria such as *Lc. lactis* subsp. *lactis* and *Leuconostoc* sp. Acetate also plays a role in the flavor of cheese, while acetoin and 2,3-butanediol are tasteless and not involved in flavor development (Monnet et al. 1995). Some bacterial cultures also have been reported to produce exocellular polysaccharides (EPS) (Cerning et al. 1992, Bouzar et al. 1996) that can improve the texture of fermented milk products.

Fermented Dairy Products

Yogurt

Although it is not known when fermented milk (yogurt) was first consumed, it is believed that fermented milk foods predated cheese and originated about 8,000 to 10,000 BC when dairy animals were first domesticated (Fox 1987). Various writings reported the consumption of fermented milk foods. Dahi, a fermented milk from India, for instance, has been consumed for thousands of years by people on the subcontinent, while a fermented milk product from the Middle

East, leben, is mentioned in the Old Testament as a complaint of the way God treated the ailing Job (Job 10:10).

The popularity of yogurt has gained new momentum in the last few decades, as more and more scientific research evidence for its health benefits are reported. In early 1900s, Élie Metchnikoff proposed that lactic acid bacteria in fermented milk must have an antibacterial effect on the putrefying bacteria of the gut by preventing the breeding of bacteria that prefer an alkaline environment. This proposal was based on his observation that people who consumed yogurt regularly in his native Balkan regions lived longer (Metchnikoff 1921, Van de Water 2003). This knowledge of the health benefits of yogurt has helped to increase yogurt consumption. The trend is also enhanced by the increased palatability of yogurt due to the addition of sweeteners and fruits (Van de Water 2003).

Yogurt can be defined simply as a dairy product from fermentation of milk. This fermentation produces lactic acid from lactose that, combined with milk protein, gives yogurt its characteristic acidic taste and texture. Yogurt is further defined in the United States as an acidic-gelled product from culturing of cream, milk, partially skim milk, or skim milk either alone or in combination by *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus.* Other ingredients can be added to alter its total solid, taste, and nutritive values (FDA 2008). In Australia, yogurt is referred to as a fermented milk product in which lactic-acid-producing microorganisms are used to ferment the dairy ingredients and other additional ingredients, and the microorganisms used must remain viable in the final product (FSANZ 2007).

Yogurt is usually made through fermentation by thermophilic microorganisms at a relatively high temperature (slightly higher than 45°C; 113°F)(Walstra et al. 2006) and a shorter fermentation time (5 hours or longer), at which the maximum pH of 4.5 is used as a guide to stop the fermentation. Therefore, this limitation is used to distinguish yogurt from other fermented milk products. The term yogurt is also be used to cover strained products from thermophilic fermentation of milk such as yogurt cheese, Greek yogurt, and other yogurt with cheese-like textures that retains the acidic characteristics of yogurt.

Yogurt Characteristics

Milk fermentation culminates in a product with different textures ranging from liquid such as kefir and koumis to semi-solid or solid such as yogurt (Van de Water 2003). Milk fermentation with LAB also results in products with distinctive tastes and aromas. LAB metabolism and the interaction between selected strains produces lactic acid and other compounds as well as coagulates milk protein. Fermentation conditions such as temperature, pH, the presence of oxygen, and milk composition also contribute to the characteristic of the final product (Nakasawa and Hosono 1992).

Certain polysaccharides, called exopolysacharides (EPSs), are also produced by bacterial culture such as *Str. thermophilus*, *Lb. delbruekii* subsp. *bulgaricus*, and other LAB such as *Lc. cremoris* and some strains of *Leoconostoc*. These polysaccharides contribute to the texture of the final product by increasing the viscosity or creating a ropy texture. Lactic acid gives a slightly tart-like taste, while acetaldehyde gives green apple flavor to yogurt (Cerning 1990, Nakasawa and Hosono 1992, Marshall 1993).

Yogurt Formulations

As an ancient food from almost every part of the world, there are many variations in the way yogurt is made, including the kinds of milk, cultures, and other added ingredients. Cow's milk is more commonly used in yogurt manufacture; however, milk from sheep,

goats, mares, and buffalo are also used in different parts of the world.

Whole milk, partially skim milk, condensed skim milk, nonfat dry milk, whey compositions, and cream can be used to formulate and standardize various yogurt mixes. These mixes should be formulated to comply with regulations as well as meet consumer expectations. The solids content of separated milk or whole milk can also be raised to 12% and 15%, respectively, by evaporation (Nauth 2006) or mixing in milk powder or whey protein concentrate. At present, premix yogurt mixes are available in various flavors, and are ready to be used at home, the office, and in food establishments.

The milk from small ruminants such as goats and sheep (Haenlein 2001) as well as milk from mares, camels, and water buffalo is commonly used in the Balkan, Middle Eastern, Indian subcontinent, and other Asian regions for yogurt manufacture. These milks are usually processed similar to cow's milk, but they contain different casein fractions due to numerous breeds among goat and sheep as compared to the few among cows. The quantities of casein in decreasing order of these milks are cow, sheep, buffalo, and goat for minor caseins. For κ-casein: buffalo, goat's, cow's, and sheep's milk; for β-casein: goat's, sheep's, cow's, and buffalo milk; for α_s-casein: sheep's, buffalo, cow's, and goat's milk (Tamime and Robinson 2007).

The starter cultures used in commercial manufacture of yogurt consist of a mixture of lactic acid bacteria such as *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus.* However, other cultures are also used in yogurt manufacture. In *dahi* preparation, for instance, *Str. thermophilus*, *Lc. lactis* biovar *diacetylactis*, and *Lc. lactis* subsp. *cremoris* are used (Tamime and Marshall 1997), while *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* species are used in probiotic yogurts either as single or mixed cultures (Tamime et al. 2005, Tamime et al. 2007). Different cultures are used in yogurt manufacture to achieve desired flavor characteristics of the product, especially lactate, aroma compounds (acetaldehyde, acetoin, and diacetyl), and texture (through production of exopolysaccharides (EPS), as well as to provide wider varieties of health products (Tamime and Robinson 2007).

Fruits and sweetening agents are usually added to enhance the versatility of taste, color, and texture of yogurt (O'Rell and Chandan 2006). The level of sweetener or fruit added to the yogurt mix depends on the brix of the fruit or the flavoring ingredients and the level of sweetness in the finished products. These sweeteners include saccharin, aspartame, Neotame™, Acesulfame-*K*®, sucralose, corn sweeteners, and maltodextrins. They are either solely added or added as a mixture of two or more sweeteners (Chandan and O'Rell 2006a). The addition of these artificial sweeteners may affect the culture growth, product quality, and consumer acceptance.

Stabilizers such as gelatin, whey protein concentrate, and various food gums are normally added to yogurt mixes to enhance and maintain the desirable characteristics of yogurt such as body and texture, viscosity and consistency, appearance, and mouth feel. During yogurt manufacture, the yogurt coagulum is often subjected to mechanical treatments such as stirring, pumping to the cooler, mixing with other ingredient such as fruits and flavors, and post-fermentation heat treatment in the case of pasteurized, ultra-high-temperature (UHT), and long-life yogurt. These treatments may cause the product to become less viscous or even show whey separation; hence, the addition of stabilizers is to overcome these problems (Tamime and Robinson 2007). A combination of several stabilizers is usually employed to avoid defects that may result from the use of a single stabilizer (Nauth 2006).

Yogurt Manufacture

Yogurt manufacture includes various key steps such as standarization of milk fat, fortification of milk solids, homogenization,

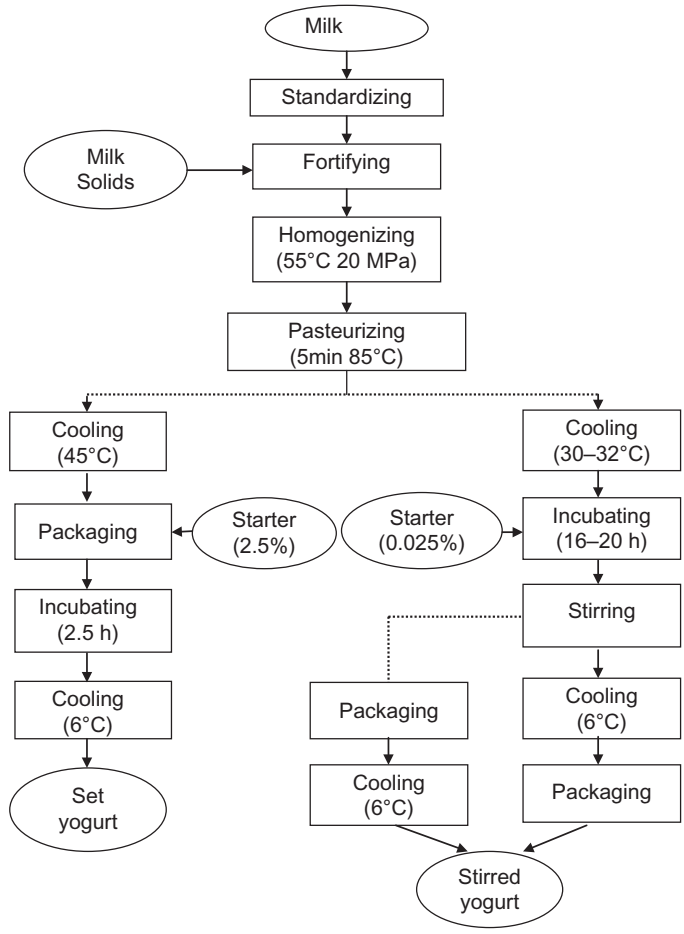

Figure 13.1. Flow process chart for set and stirred yogurt manufacture. Adapted from Walstra et al. (2006).

heat treatment, inoculation and fermentation, mixing of fruits and sweeteners, packaging, and storage (Figure 13.1). Standardization of milk fat is essential in yogurt manufacture due to variations in the fat content of milk sourced from different breeds and seasons as well as to comply with regulations in particular countries. This step is carried out by pumping whole milk into a milk separator to remove excess fat and produce skim milk with a certain fat content to conform to standards in different countries (Tamime and Robinson 2007).

Milk solids also are fortified to comply with the regulations of various countries concerned and to prevent whey separation. The milk solids can be raised by boiling milk to evaporate the water, adding milk powder, and concentrating the milk membranes. Boiling is traditional; however, it is not cost effective because it uses excess steam in processing. Addition of milk powder, usually skim milk powder, is incorporated into either whole milk, skim milk, or water, and then mixed thoroughly. A variety of mixers or blenders are avalable for this purpose. They are designed to provide complete dispersion of the dry milk powder into an aquoeus phase, complete hydration of the dry ingredient so the residual lump produced is minimal,

minimize air incorporation to reduce possible foaming, and enable easy cleaning and sanitation of the unit (Tamime and Robinson 2007).

Milk evaporation is normally carried out under vacuum conditions and at a high temperature to avoid damage to the milk constituents while removing the desired amount of water. Membrane concentration of milk, either whole or skim, is an alternative method. This is acchieved by ultrafiltration or reverse osmosis. Milk constituents that pass through the membrane are called the permeate, and the constituents that do not pass through the membrane are called retentate (Tamime and Robinson 2007).

The yogurt mix is heat treated to destroy and/or eliminate pathogens and other vegetative cells, produce factors stimulatory or inhibitory to the yogurt starter culture, and change the physicochemical properties of milk constituents that are relevant to yogurt making (Chandan and O'Rell 2006b). Severe and extensive heat treatment of milk can cleave the calcium phosphate complexes with casein micelle, resulting in destabilization, aggregation, and precipitation (Chandan 2006). This heat treatment and the resulting interactions improve the water binding capacity of the protein system (Nauth 2006).

Inoculation and fermentation of the yogurt mix is the fundamental step that gives yogurt its final characteristics. After heat treatment, the yogurt mix is cooled to between 40°C and 45°C (104°F and 113°F), the optimum temperature for mixed starter culture (Lucey 2004). The starter culture can vary from 0.5% to 6%, depending of type of yogurt and fermentation system. Incubation or fermentation of yogurt mix can be carried out for as little as 2 hours when inoculated with 5% starter and at 43°C to 45°C (109°F to 113°F) to more than 6 hours if inoculated with 0.5% to 1.5% starter (Nauth 2006). The fermentation time can be longer, 16 to 18 hours, if the mix is incubated at 30°C (86°F) with 3% inoculum (Tamime and Robinson 2007). The fermentation is terminated when pH reaches 4.4 to 4.5. Fruits can be incorporated into the bottom of cups (set-style yogurt) and the inoculated yogurt mix added to the top followed by fermentation, or after fermentation (stirred-style yogurt) by mixing the fruit preparation with plain yogurt (Nauth 2006).

Physicochemical Changes During Yogurt Manufacture

During fermentation, certain physicochemical changes take place to the milk constituents that alter the properties of the yogurt mix and give the characteristics of the final product. These changes include acidity, viscosity, and, to a lesser extent, flavor. The increase in acidity is mainly due to the production of lactic acid and, to a lesser extent, acetic acid from lactose hydrolysis through the homofermentative pathway (Cogan 1995, Van de Water 2003). Increasing viscosity results from the destabilization of the casein complex, when the casein micelles flocculate at or near their isoelectric point and the colloidal calcium phosphate partially solubilizes due to increased acidity (Nauth 2006). The result of this destabilization is an irreversible gel (Tamime and Robinson 2007) that is responsible for the texture of yogurt.

Flavor development also takes place during yogurt manufacture. The flavor compounds in yogurt consist mainly of acetaldehyde and diacetyl. Acetaldehyde is produced from the amino acid threonine in milk as well as threonine produced by the lactobacilli during the proteolysis of milk proteins. Diacetyl is produced during yogurt fermentation by *Str. thermophilus* and, to a lesser extent, *Lb. delbrueckii ssp. Bulgaricus,* probably in a manner similar to the mechanism of diacetyl production in soft cheeses such as quark. Because yogurt starter culture does not metabolize citric acid, the precursor of diacetyl, i.e., pyruvic acid, is formed during sugar fermentation (Walstra et al. 2006).

Quality of Yogurt

The quality of yogurt, just as for any other food, is essential to assure safety for human consumption, comply with regulations, attain a specific shelf life, and achieve high organoleptic standards within existing constraints of manufacture and marketing. Therefore, it is important to ensure the quality of all ingredients at the time of receiving, handling, and manufacturing so the end product complies with certain chemical, microbiological, and organoleptic standards. In large scale manufacture, the hazard analysis and critical control point (HACCP) concept is employed to monitor all aspects of the raw material, equipment, manufacturing process, and end product.

The quality of liquid milk, whole or skim, as a raw material is essential for yogurt manufacture. Various tests to determine the total solids, fat, protein, antibiotics, taints, organochlorine, organophosphate, and dirt contents are usually performed. If milk powder is the raw material, then tests for its solubility are also important, in addition to the microbiological tests and moisture content of skim milk (Tamime and Robinson 2007).

The starter culture added to the yogurt mix is usually in a liquid form containing 1:1 (chain:chain) ratio of *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus.* In this case, checking the balance before application is required to confirm that the culture is suitable for use (Tamime and Robinson 2007). If the culture is for probiotic yogurt, the direct-vat inoculation (DVI) of preserved cultures is preferable due to difficulties in maintaining the correct ratio of the probiotic bacteria if they are grown as a mixed culture prior to inoculation (Tamime et al. 2005).

Fruit must be incorporated into yogurt using sterile equipment to avoid yeast and mold contamination. The blending and filler areas are very crucial to the microbiological quality of the yogurt with respect to yeast and mold. These areas should have high-efficiency-particulate-air (HEPA) -filtered air to keep out airborne yeast, mold, and other contaminants (Nauth 2006). The containers used must be free from possible contaminants in the final stages of packaging and storage. The yogurt must be stored at a refrigerated temperature as soon as the packaging process is completed.

Shelf Life of Yogurt

The shelf life of yogurt at a refrigerated temperature is around 34 weeks, depending on the standard hygiene observed during manufacture and the microbial quality of the ingredients and the packaging material. Various techniques have been used to extend yogurt's shelf life, including freezing and drying, gas flushing, adding preservatives, using aseptic equipment, applying multiple frequency microwave, and sterilizing with heat (Tamime and Robinson 2007).

The shelf life of Greek yogurt and/or labneh is quite short. At refrigeration temperature, a shelf life of seven to 10 days is recommended (Yamani and Abu-jaber 1994). The relatively short shelf life of cloth bag labneh is largely responsible for the wide use of benzoates and sorbates to control growth of spoilage microorganisms (Mihyar et al. 1999) due to aerial contamination during sun drying or pressing, although the latter can be minimized. The shelf life of yogurt cheese is similar to that of labneh anbaris, which is longer than that of labneh due to a higher total solids content and more concentrated lactic acid. At ambient temperature, the shelf life is about 12 to 18 months as long as the product is kept submerged under olive oil (Tamime and Robinson 2007).

Application of Yogurt

The popularity of yogurt has increased over time from simply plain fermented milk to fruit-added and flavored yogurts. At present, fruit-added yogurts are the most popular

yogurt available for sale. However, yogurts flavored with various ingredients are also being marketed in an attempt to improve yogurt consumption. The following are some uses of yogurt as an ingredient.

Salad Dressing

Yogurt-based salad dressings and dipping sauces are well documented. These include salad dressing containing salt, spices, dried onion, garlic, and parsley (Stainberg 1983b) and yogurt dip containing onion, clam, cheddar, and blue cheese (Stainberg 1983a). Other yogurt dressings include those containing honey, Dijon mustard, and celery seed; chopped fresh cilantro, lemon juice, cumin, and sweet chili sauce; and low-fat yogurt dressing containing lemon juice, Dijon mustard, fresh parsley, and fresh chives (http://www.allrecipes.com).

Yogurt Cheese

Yogurt cheese is a strained yogurt similar to labneh anbaris. Its preparation includes heating milk (whole or skim) to 70°C (158°F) and cooling it to 46°C (83°F), followed by yogurt culture inoculation and stirring. The mixture is cooled down further to 30°C (86°F) without agitation, then annatto, rennet, and starter culture containing *Lc. lactis* subsp. *lactis* and subsp. *cremoris* are stirred in for two to three minutes. After two to three hours of incubation, the coagulum formed is coarsely cut (2 to 3 cm), scooped into a cloth bag, and the whey drained for 24 hours at 20°C to 25°C (36°F to 77°F). Further draining of whey at 5°C to 10°C (41°F to 50°F) for 24 hours is carried out before packing and storing at a refrigerated temperature. Optionally, sorbate and salt can be added to the curd before packing (Tamime and Robinson 2007).

Frozen Yogurt

Frozen yogurt is a yogurt base mix with various additional ingredients such as fruit syrup and stabilizer or emulsifier. Frozen yogurts can be classified into three groups: soft, hard, and mousse. The product resembles ice cream with the characteristic sharp acid taste of yogurt and coldness of ice cream. The high level of sugar and emulsifier or stabilizer added to the product is necessary to maintain the air bubble structure during the freezing process (Tamime and Robinson 2007).

Soft frozen yogurt is made by mixing a cold yogurt base (80%) with a 20% fruit syrup base and stabilizer or emulsifier, and then freezing in an ice cream freezer at −6°C (21°F). The frozen yogurt is then packed and stored at 0ºC (32ºF0 to −6ºC (21°F). Hard frozen yogurt is made in a similar manner but with a different composition of yogurt and fruit base syrup (56%:35%, respectively). Hard frozen yogurt is stored at −25°C (−13°F) rather than −6°C to 0°C (21°F to 32°F). Mousse-type frozen yogurt is made my mixing yogurt with a hot mousse mix containing skim milk, sugar, and stabilizer or emulsifier; cooling and whipping in an ice cream freezer; packing; and then storing at 0°C (32°F) (Kosikowski 1981, Tamime and Robinson 2007).

Greek Yogurt

The term Greek yogurt or Greek-style yogurt used in Europe refers to strained yogurt such as labneh. It is produced in many countries in the Balkans, eastern Mediterranean, Turkestan, and the Indian subcontinent, and is called by different names. Strained yogurt is a semi-solid product containing 23 to 25 g total solids/100 g, of which 8 to 11 g/100 g are fat (Tamime and Robinson 2007). Traditionally, labneh is produced by straining cold unsweetened yogurt in a cloth bag, animal skin, or earthenware vessel (Ibrahim et al. 1999). In large-scale production, strained yogurt is produced using large cloth bags, mechanical separators, ultrafiltration, and product formulation (Tamime and Robinson 2007).

Strained yogurt is a traditional food with a cheese-like texture but the specific acidic taste of yogurt. It may be considered as an intermediate between conventional fermented milks and high-moisture, unripened soft cheese such as quark (Varnam and Sutherland 2001). It is usually consumed with bread as part of the main meal (Tait 2005). In Greece, strained yogurt is made mainly from sheep or goat milk and is used as a base for preparation of a dip called tzatziki, a mixture of strained yogurt with olive oil, cucumber, garlic, salt, pepper, dill, and sometimes lemon juice, parsley, and mint.

Yogurt Bars

Yogurt bars are various products that contain yogurt, dried yogurt, or frozen yogurt. They include cereal yogurt bars, frozen yogurt bars, and probiotic yogurt bars. Cereal yogurt bars are mostly yogurt-topped or yogurt-coated cereal or muesli bars (O'Donnell 2002, Anon 2002) that come in various flavors. The manufacturing steps of these bars include baking of the yogurt-topped or coated bars; hence, dried products are obtained (www.recipes.eu.com/recepi92.html) that can be stored at ambient temperatures. The yogurt used for this purpose can either be plain or powdered. Other ingredients such as egg and flour are added to increase viscosity and aid drying. Dried powdered yogurt offers advantages over plain yogurt because it has a longer shelf life and is aimed toward the do-it-yourself market, baby food manufacturers, and food and baking industry (Tamime 2003).

Frozen yogurt bars are yogurt-coated products of various flavors with cereal coating. Low-fat yogurt and sorbet with fruit flavors coated with cereal or sandwiched between wafers are available (Anon 2006). Yogurt containing active yogurt cultures as well as probiotic cultures has also been incorporated into bars, either dried or frozen. The probiotic yogurt bars contain more than 10 billion probiotics and are also a source of prebiotics. In addition, they contain whole grains, nuts, and dried fruits (Anon 2008).

Cottage Cheese

Cottage cheese is a fresh, particulate, and slightly acidic cheese made by fermentation of skim milk using lactic-acid-producing bacteria. The flavor of cottage cheese ranges from bland to sharp, with overtones of diacetyl (Kosikowski and Mistry 1997). In the United States, cottage cheese is made by mixing cottage cheese dry curd with a creaming mixture consisting of milk or other milk derivatives. The final products must contain not less than 4% (by weight) milk fat and not more than 80% moisture content. The cottage cheese dry curd consists of one or more of the following dairy ingredients: sweet skim milk, concentrated skim milk, and nonfat dry milk. Harmless lactic-acid-producing bacteria with or without rennet and/or other suitable enzymes are added to coagulate the mixture until the pH reaches 4.5 to 4.7 (FDA 2008). Salt and fresh or cultured cream is generally added to the cheese. Distinctive features of the product are its granular form and, despite its low fat content, its creamy flavor (Walstra et al. 2006).

Cottage Cheese Formulation

The main ingredient in cottage cheese manufacture is skim milk. The lactic-acid-producing bacteria used include *Lc. lactis* subsp. *lactis* or *Lc. lactis* subsp. *cremoris* as the main acid producers and *Leuc. mesenteroides* subsp. *cremoris* as the main flavor producers. A little rennet containing chymosin is usually added to enhance whey expulsion during heating and to improve texture and firmness (Kosikowski and Mistry 1997). The concentration of culture is 5% to 6% for a short-set process or 0.25% to 1% for a long-set process (Walstra et al. 2006).

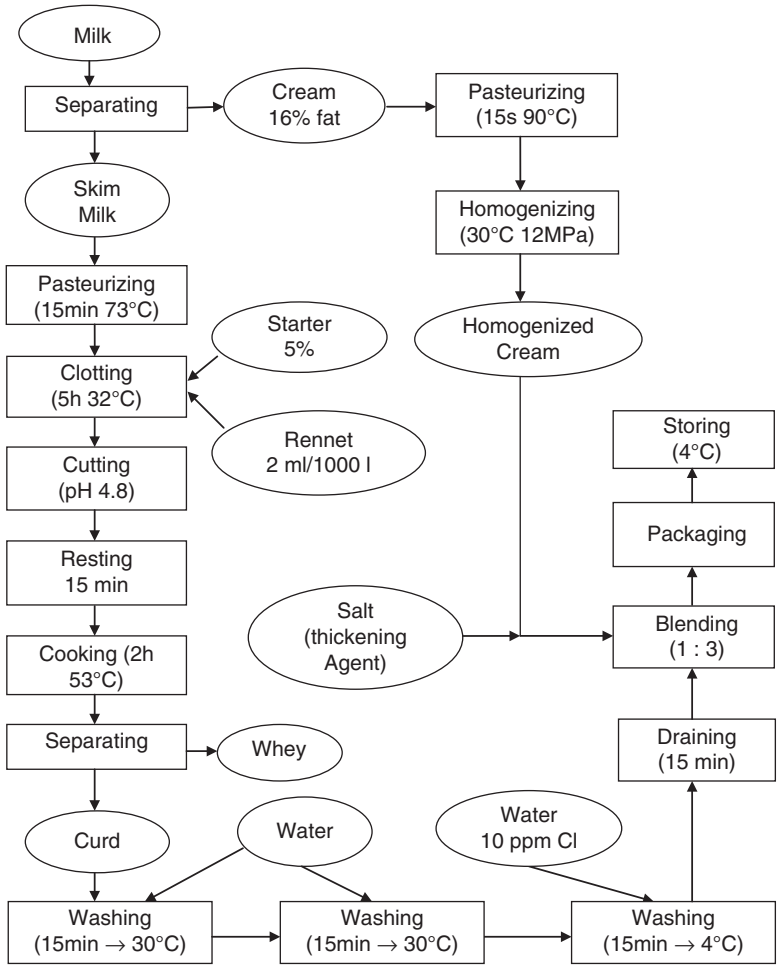

Figure 13.2. Flow process chart for cottage cheese manufacture. Adapted from Walstra et al. (2006).

Cottage Cheese Manufacture

The traditional short-set manufacturing process of cottage cheese is as shown in Figure 13.2. Pasteurized skim milk is heated to 32°C (58°F) and mixed with starter culture and rennet. The starter culture is a non-gas-forming culture to prevent the cut curd from floating. The rennet can be incorporated at the same time as the culture or after 1 to 1.5 hours of incubation with starter. The formed curd is then cut into cubes, rather than stirred, when the pH reaches 4.6 to 4.8. The cubed curd is allowed to rest for 15 minutes, following by cooking at 53°C (127°F) 2 hours with gentle stirring. Whey separates during cooking and the curd becomes firmer. The cooked curd is then washed several times to remove lactic acid and cool the curd. After draining, the washed curd is blended with pasteurized homogenized cream, and the cottage cheese is packed and stored (Walstra et al. 2006).

Direct acidification of the milk with inorganic or organic acid is an alternative to starter culture acidification. Acid anhydrides

such as mesolactide and glucono-δ-lactone as well as inorganic acids such as phosphoric and hydrochloric acids have been used (Kosikowski and Mistry 1997). Acidification is carried out at a low temperature with vigorous stirring of the milk until the pH reaches 4.6. Heating is carried out by passing an electric current through the milk without agitation. The milk is then allowed to set for about 12 minutes. The remaining manufacturing steps are the same as in the short-set method, above (Walstra et al. 2006).

Quality of Cottage Cheese

The appearance and flavor perception of cottage cheese are directly affected by the curd size and their distribution. The freshly cut curd is soft and fragile. Leaving the cut curd for 10 to 15 minutes drains out a little whey; hence, increasing the firmness of the curd granules and preventing damage during stirring. Slower cooking of the curd results in more even syneresis, whereas faster heating produces grainy curd with a dry, firm rind. Therefore, the rate of heating affects the consistency and firmness of cottage cheese (Walstra et al. 2006). Heating at a higher temperature terminates the activity of many bacteria and deactivates the rennet.

Curd washing is typical in cottage cheese manufacture. Insufficient washing results in a product with a rather acidic flavor, rendering it unacceptable. The curd is washed three times by pumping in cold water equal to the amount of whey drawn off, and 5 to 20 ppm of active chloride is often added to the last wash water to prevent growth of undesirable microorganisms (Kosikowski and Mistry 1997, Walstra et al. 2006).

Adding cream (sweet or cultured) to the low-fat curd improves the cottage cheese flavor and texture because it replaces the fat that is lost during manufacture and lubricates the curd granules. The pH of the cream plays important role in determining the quality of the cottage cheese. Well-soured cream with a low pH reduces liquid separation because it does not separate into free cream (Walstra et al. 2006). Salt can be incorporated into the cream before mixing with the curd or added separately during mixing of the curd and cream (Kosikowski and Mistry 1997).

Shelf Life of Cottage Cheese

The shelf life of cottage cheese is quite limited because its composition permits microorganisms to grow (Walstra et al. 2006). When refrigerated, the shelf life of most commercial cottage cheese is about 12 days, though there are products manufactured under stringent quality controlled conditions that can have a shelf life up to 21 days. The defects of cottage cheese include lack of fine flavor; high acid; bitterness; grainy-mealy, pasty, or weak-soft texture; over-creamed, shattered curd; and free whey appearance (Kosikowski and Mistry 1997).

Attempts to increase the shelf life of cottage cheese include the addition of sorbic acid to the creaming mix. Cottage cheese also may be packaged under carbon dioxide to inhibit the growth of undesirable bacteria, especially the Gram-negative psychrotrophs (Walstra et al. 2006).

Use of Cottage Cheese

Cottage cheese can be eaten directly, with fruits, with fruit puree, with green salad, on toast as a spread, and as an ingredient in many different foods. Its use as a filling is notable in products such as blintzes, a pancake-like food originating in Eastern Europe that is associated with Jews during Hanukkah. Cottage cheese mixed with ricotta cheese, mozzarella cheese, and spinach in lasagna. It can be combined with quark and used in cheesecake. In India, sandesh, a cotztage cheese analog, is mixed with 10% herbal paste containing turmeric, coriander,

curry leaf, spinach, and aonla as sources of antioxidants to make herbal sandesh, a value-added health food (Bandyopadhyay et al. 2007).

Quark

Quark is a natural fresh, soft cheese made from skim milk with the specific organoleptic characteristics of a milky white color, soft body, smooth texture, good spreadability, no appearance of water or whey on its surface, and a clean but mild acidic flavor. Because it is made from skim milk, quark is a casein-coagulated, low-fat fresh cheese with a high moisture content (Winwood 1983). Quark, also known as quarq, originated from bag cheese, and it is still produced in that way sometimes (Walstra et al. 2006). It originated from central Europe and is prepared in bag with different shapes such as the traditional wedge shape of Polish twaróg.

Quark Formulation

Pasteurized skim milk is the raw material in the basic production of quark. Lactic-acid-producing bacteria such as *Str. lactis* or *Str. cremoris* are usually used. Flavor-producing organisms such as *Str. diacetylactis* also can be used (Winwood 1983). The starter is 1% to 2% (Walstra et al. 2006, Farkye 2007); however, 5% starter can be used to reduce the fermentation time to about 5 to 6 hours (Winwood 1983).

In recent decades other cultures have been used, including yogurt, acidophilus and buttermilk cultures. The yogurt culture consists of *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulagaricus* isolated from commercial yogurt. The acidophilus strain consists of *Lb. acidophilus*, and the buttermilk culture consists of *Str. lactis*, *Str. cremoris*, and *Leuc. cremoris* (Shah et al. 1990). Rennet is sometimes added to produce a firmer product (Walstra et al. 2006), although it may cause bitterness (Sohal et al. 1988).

Quark Manufacture

The basic production procedure for quark cheese is similar to that of cottage cheese except that quark manufacture includes a pre-acidification step, whereas cottage cheese manufacture includes washing the curd. The procedure includes skim milk preparation, pasteurization, culturing and fermentation, whey separation, and creaming of the quark produced. Skimming of whole milk is aimed to separate 40% of its butterfat leaving skim milk containing less than 0.05% butterfat (Winwood 1983). Pasteurization of the skim milk at 72°C (162°F) for 15 seconds followed by cooling to 22°C to 23°C (72°F to 73°F) is particularly important because quark, like cottage cheese, is a high-moisture soft, fresh cheese.

The milk is inoculated with 1% to 2% bulk starter culture consisting of lactic-acid-producing bacteria, *Str. lactis* or *Str. cremoris* (Winwood 1983). Flavor-producing starter such as *Str. diacetylactis* is commonly used for the release of flavor (Walstra et al. 2006). Incubation of the mix is carried out at 22°C to 23°C (72°F to 73°F) for 16 to 18 hours (Winwood 1983), although this process may be conducted at a higher temperature (Walstra et al. 2006) of 30°C (86°C) with 5% starter inoculum to speed up the fermentation. Rennet (approximately 0.5% to 2% of the skim milk) usually is added 60 minutes after inoculation with starter cultures (Winwood 1983, Farkye 2007). Rennet enhances protein destabilization by raising the pH at which a firm coagulum is formed, usually from about 4.6 to between 4.7 and 4.8. It also facilitates easy draining of whey, produces a firmer curd and better final product (Winwood 1983, Walstra et al. 2006), and increases yield (Sohal et al. 1988).

The whey is separated when the pH has dropped to a value between 4.6 and 4.7. At this pH and low temperature the curd exhibits little syneresis. The addition of a high level of rennet can cause premature syneresis,

resulting in a non-homogeneous product (Walstra et al. 2006). Traditionally, whey is separated is by cutting the curd and allowing it to drain in a linen cloth. This is time consuming, laborious, and unsuitable for large-scale production. The keeping quality of the cheese is also limited due to possible contamination. In a modern production plant, a centrifugal quark separator is used to separate the coagulated skim milk. The drained quark can then be cooled at 5°C (41°F) or lower, packed, and stored at a refrigerated temperature (Winwood 1983). This method enables the adjustment of the quark's water content by varying the flow rate of the whey, but it does not work well if the quark is produced from whole milk (Walstra et al. 2006).

The whey can also be separated by filtration followed by homogenization to produce smooth quark. Ultrafiltration and diafiltration of quark made from skim milk are alternatives to centrifugation technique; however, the latter is of little use. Ultrafiltration increases the total solids of the final quark to 17% to 19%. The quark produced from ultrafiltration of sour skim milk is rated as close to the conventional quark, with no bitter taste. High heat treatment of milk before acidification by lactic acid bacteria followed by ultrafiltration results in quark with smooth texture (Patel et al. 1986) and higher yield (Walstra et al. 2006).

Shelf Life of Quark

The shelf life of quark under pilot plant studies is limited to less than 14 days due to the growth of contaminating microorganisms (Ailsa et al. 1969); therefore quark should be kept at refrigerated temperatures and consumed within few days. Contamination by yeasts and moulds may reduce the shelf life of quark. At higher storage temperatures, flavor deterioration and syneresis occurs, hence affecting the quality (Walstra et al. 2006).

Bitter Taste of Quark

Bitter taste arising from proteolysis is another concern. This is particularly true if rennet is used in the preparation. During wheying out, most of the rennet added to the skim milk is discarded with the whey. However, traces of rennet may remain in the curd. Rennet is known to cause bitterness, especially in quark that is produced using ultrafiltrated milk before fermentation due to high calcium content in the retentate (Jelen and Renz-Scauen 1989). Rennet retention in quark increases with low pH at draining, and its activity is high in low-pH products (Guinee and Wilkinson 1992). Reducing the rennet is one way to eliminate the bitter taste, though this may reduce the yield of the cheese. The use of 388 units of rennet/1,000 kg of milk has been shown to produce acceptable quark with reduced bitterness, good yield, and extended shelf life (Sohal et al. 1988).

Use of Quark

Quark is a suitable ingredient in several dishes due to its neutral flavor, no added salt, and its consistency (smooth, can be blended) (Walstra et al. 2006). It can be used as an ingredient in cakes such as the German cheesecake käsekuchen and Dutch kwarktaart, in sandwiches as a filling or spread, and in salad.

Quark also has been used in the preparation of shrikhand, although strained yogurt is more commonly used. Several spices such as nutmeg and saffron are also used to flavor the product (Tamime and Robinson 2007).

Quark can be used to prepare foods such as healthy tiramisu; sweet quark pasta casserole; rhubarb vanilla tart; pinwheels with poppy seed, cherry, and raisin; the German poppyseed cake mohnstrietzel; vanilla plaits with raisin; and many other delicacies (www.recipezaar.com/recipe.php).

Cream Cheese

Cream cheese is a soft, uncured cheese produced from fermentation of milk, nonfat milk, or cream, either alone or in combination, by lactic-acid-producing bacteria (FDA 2008). It has an unusual microstructure owing to its peculiar method of manufacture (Monteiro et al. 2009). Cream cheese is a concentrated acid milk gel system with a variable fat content. As is the case for other dairy product groups, many low-fat and even nonfat cream cheeses have entered the market recently (Breidinger and Steffe 2001).

Cream cheese is quite similar to Neufchatel, which contains less fat and has a grainer body. The nutty flavor of both products is similar, but the intensities are different. Cream cheese is used in cheesecake, salads, dips, and savory snacks, and as a sandwich spread. The minimum fat content of cream cheese is 33% and the maximum moisture content is 55% (FDA 2008).

Cream Cheese Characteristics

Cream cheese, like Bakers' cheese, Neufchatel, and cottage cheese, uses lactic acid bacteria. However, cottage cheese manufacture includes a washing step to remove most the lactic acid produced. Cream cheese is a sweet, soft, mild-tasting unripened white cheese.

The various end uses of cream cheese determine its functional requirements. It should possess a smooth texture when used as a spread; however, gritty or grainy defects sometimes occur (Sainani et al. 2004). Firmness is also important for its use as a spread, while melting characteristics (flow, viscosity) are important for its applications in heated products such as cheesecake (Fox et al. 2000).

Cream Cheese Formulation

The raw material in cream cheese manufacture is milk, nonfat milk, or cream, either alone or in combination (FDA 2008). The fat content of cream cheese mix is 11.5%. This can be achieved by mixing portions of milk (3.8% fat) and cream (40.0% fat). The rennet extract needed for fermentation is 4.4 ml/1,000 kg of mix in addition to 5% starter culture (Kosikowski and Mistry 1997). Active commercial bulk starter culture is normally employed in cream cheese manufacture, although a 1 : 1 mixture with *Lb. delbueckii* subsp. *bulgaricus* also can be used (Covacevich and Kosikowski 1977).

Cream Cheese Manufacture

The two methods of cream cheese manufacture are cold-pack and the hot-pack. In the cold-pack method, the mixture of milk and cream is blended, pasteurized, and homogenized. The homogenized mix is then cooled to 31°C (88°F) before the addition of starter culture. The fermentation takes place and acid curd with desirable flavor is produced. The fermentation is terminated, when the pH of the mix reaches 4.7, by slightly heating the curd in water. The curd is then cooled and drained in nylon or muslin bags. After the draining, the curd is simply mixed with salt and stabilizer and kneaded (Kosikowski and Mistry 1997).

In the hot-pack method, the same procedures as in the cold-pack method are followed; however, after draining, the dry unkneaded curd is cooked in pasteurization vats or kettles with heavy duty agitators. During this time salt, a stabilizing agent, and gums are added. Next, the mixture is pasteurized, homogenized, pumped into packages, and cooled in sealed forms. A centrifuge is commonly employed to concentrate the hot curd from whey, offering an advantage over the use of nylon or muslin bags (Kosikowski and Mistry 1997). The whole process of cream cheese manufacture results in disruption and reconfiguration of the typical continuous three-dimensional casein matrix structure that characterizes most cheeses (Sanchez et al. 1996).

Quality of Cream Cheese

Cream cheese manufacture, as in other fresh acid-curd cheese varieties, involves pretreatment of the raw material (standardization, pasteurization, and perhaps homogenization), slow quiescent acidification, gel formation, drying of gel (whey separation), and in some cases further treatment of the curd (pasteurization, cutting, addition of salt, stabilizers, and homogenization) (Fox et al. 2000). Acidification is arguably the crucial step that determines the quality of the final product.

Acidification slowly converts lactose into lactic acid by the starter culture, resulting in gel formation (Fox et al. 2000). Cream cheese firmness is strongly influenced by the pH. Adjusting the pH of the cheese upward or downward by exposing the finished cheese to an atmosphere of a volatile base or acid, such as ammonia or acetic acid, changes its firmness. The cheese firmness decreases significantly as the pH is increased (by exposure to ammonia vapor) from an initial value of about 4.6 to 6.3 (Almena-Aliste and Kindstedt 2005). This pH-induced change in firmness is reversible (Almena-Aliste et al. 2006). Hence, altering the pH of cream cheese provides a product with desired the firmness.

The water holding capacity of the cheese is also directly and substantially affected by pH. However, the viscosity of the serum phase and the distribution of calcium between the casein-associated and soluble states are not affected. This suggests that the observed changes in firmness and water holding capacity are caused by pH-induced changes in casein-to-water interactions, and not by changes in stabilizer function or calcium distribution (Almena-Aliste et al. 2006).

Shelf Life of Cream Cheese

The shelf life of cream cheese is relatively short. The fresh, cold-pack cheese displays a fine aromatic flavor pleasing to many consumers and suited for baking of cheesecake. However, the cheese deteriorates quickly under refrigerated conditions between one and two weeks, producing yeasty, fruity, and moldy defects (Kosikowski and Mistry 1997). The hot-pack cheese has a longer shelf life due to the heat treatment of the curd. Under refrigerated conditions the shelf life of hot-pack cream cheese is about three months (Fox et al. 2000).

Use of Cream Cheese

Cream cheese's characteristics such as smooth texture, spreadability, and mild taste can be conveniently covered with other ingredients such as fruit puree to produce a rich, spreadable mix with the desired flavor. Cream cheese is often spread on toast, bagels, and crackers. It is an ingredient in dips with various other ingredients such as herbs, fruits, nuts, and spices (Teubner 1998), which are good companions for other snacks and wine. Cream cheese is used in baking, such as in preparation of rugelach, or cream cheese crescent, and cream cheese pastry. Rugelach is a traditional Jewish delicacy containing raisins and walnuts that is served during Hanukkah. It is used in pies and tarts with a wide selection of fillings. In cheesecake preparation, cream cheese is used either alone or in combination with cottage cheese and sour cream (Horn et al. 1997).

Cultured Buttermilk

Cultured butter milk is, by far, the replacement of conventional buttermilk (Walstra et al. 2006). Conventional buttermilk is widely used in the food industry because of its emulsifying capacity and its positive impact on flavor. Commercial buttermilk is sweet buttermilk, a byproduct from churning sweet cream into butter (Sodini et al. 2006). It is the aqueous phase that is released during the churning, and it contains all of the water-soluble components of cream such as milk

protein, lactose, and minerals. It also includes material derived from the milk fat globule membrane (MFGM), which is disrupted during the churning and mostly migrates to the buttermilk fraction (Corredig and Dalgleish 1997).

Buttermilk contains phospholipids, which are very sensitive to autoxidation due to a high concentration of polyunsaturated fatty acid. Therefore, buttermilk can easily develop an off-flavor, often called metallic, which can become quite pungent. The shelf life of buttermilk is comparably short, especially for buttermilk with high-fat cream content, even if antioxidants such as ascorbic acid have been added. Cultured buttermilk, on the other hand, is a product from mesophilic acid bacteria fermentation of pasteurized skim milk that possess a mild acidic taste with an aromatic diacetyl flavor and a smooth viscous texture (Walstra et al. 2006). It contains less phospholipid and has a longer shelf life.

Cultured Buttermilk Formulations

Unlike conventional buttermilk, cultured buttermilk uses pasteurized skim milk or homogenized, pasteurized low-fat milk that contains less than 1% fat (Walstra et al. 2006). Addition of milk-solids-not-fat (MSNF) and partly skim milk containing 0.7% to 2% fat also is common (Kristoffersen and Gould 1966).

Starter culture containing *Lc. lactis* subsp. *cremoris* and *lactis*, *Lc. lactis* subsp. *lactis* biovar. *Diacetylactis,* and *Leuc. mesenteroides* subsp. *cremoris* are used for lactose fermentation. *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* also may be included in the starter culture. The first two species are known as lactic acid producers, thus they are responsible for the production of lactic acid. The latter two species are known as the aroma producers; they produce aromatic compounds, mostly diacetyl and acetaldehyde, which are responsible for the characteristic aroma of cultured buttermilk. The level of aroma-producing bacteria in the starter culture mix should not exceed 20% in order to produce cultured buttermilk with a balanced mix of aroma and lactic acid. The acetaldehyde content of cultured buttermilk should not exceed 1 mg/kg because it will give a yogurt-like aroma, considered a defect in buttermilk. This correct level be achieved by using *Leuc. mesentorides* subsp. *cremoris* instead of *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* (Walstra et al. 2006).

Manufacture of Cultured Buttermilk

Cultured buttermilk is made from pasteurized skim milk or homogenized, pasteurized low-fat milk, usually containing less than 1% fat (Walstra et al. 2006). After high temperature pasteurization the milk is cooled to 22°C (72°F) and inoculated with about 1% to 3% mesophilic starter. The milk is fermented at 19°C to 22°C (66°F to 72°F) for 15 to 20 hours until a pH of 4.6 to 4.7 is reached. The coagulum is formed and stirred slowly and the resulting product is cooled and packaged. Fruit condiments, essences, and butter flakes may be added to the plain cultured buttermilk (Kosikowski and Mistry 1997, Walstra et al. 2006).

Cultured buttermilk manufacture is characterized by the use of low-fat milk, high temperature pasteurization, lactic acid fermentation, and quick refrigeration of the final product. The milk is pasteurized at about 85°C (185°F) for 30 minutes, which increases the viscosity of the product, prevents wheying-off, permits rapid growth of cultured microorganisms, and destroys bacteriophages and other undesirable bacteria (Kosikowski and Mistry 1997). Lactic acid fermentation of the raw material results in a smooth and fairly thick body due to the coagulation of milk proteins, and the aroma produced by the fermentation of citric acid and lactose. The texture varies according to the total solids of the raw material (Walstra et al. 2006).

Quality of Cultured Buttermilk

Cultured buttermilk may have defects in flavor and texture. Over acidification during storage is an occasional problem, but lack of flavor caused by reduction of diacetyl to acetoin is more frequent (Monnet et al. 1995). Unclean, putrid, and bitter flavors are also problems associated with cultured buttermilk. Unclean flavor is an indication of an unclean raw material that may contain *Escherichia*, *Aerobacter*, and psychrotrophic bacteria such as *Pseudomonas*. The bitter taste is derived from improper pasteurization of milk, rendering limited deactivation of proteolytic enzymes (Kosikowski and Mistry 1997). High acid and metallic flavors are also problems associated with the quality of cultured buttermilk. The high acid flavor is due to continual growth of starter culture, thus producing more lactic acid. This problem can be solved by adding a small amount of starter culture and monitoring the acidity closely. The metallic flavor results from the chemical reaction of protein and fat oxidation catalyses by copper contamination. Using stainless steel or glass equipment during manufacture and storage can limit this development (Kosikowski and Mistry 1997).

Curd floating and wheying off are associated with the texture defects in cultured buttermilk. Curd floating is due to the production of carbon dioxide by citric-acid-fermenting-bacteria (Walstra et al. 2006). Wheying off is a phenomenon in which water is separated from the curd. Although the separation does not affect the nutritional value of buttermilk, it is aesthetically objectionable (Kosikowski and Mistry 1997, Walstra et al. 2006). This problem can be solved by adding thickening agents such as pectin (Walstra et al. 2006).

Shelf Life of Cultured Buttermilk

At refrigerated temperature, the keeping quality of cultured buttermilk is 2 to 3 weeks (Walstra et al. 2006). A similar shelf life of five to 21 days is reported for most cultured buttermilk sold in the United States (Kristoffersen and Gould 1966).

Use of Cultured Buttermilk

The commercial use of buttermilk is mainly in the baking and dairy industries and in prepared dry mixes (IDFA 2008). Other uses of cultured buttermilk include plain or flavored beverages, salads or salad dressings (Kosikowski and Mistry 1997), sauces, marinades, soups, milk substitute (Scarpa 1997), waffles, and blintzes (Horn et al. 1997).

In baking, cultured buttermilk is used in biscuits, pancakes (Horn et al. 1997), breads, cookies, and cakes. In the dairy industry, it is an ingredient in the manufacture of recombined milks (Singh and Tokley 1990), cheese (Joshi et al. 1994), ice cream (Chandan 1997), and yogurt (Trachoo and Mistry 1998). In ice cream preparation, buttermilk helps to smooth the texture and prevent ice crystals. It also adds a richness of flavor, despite its low fat content. Buttermilk also can be used as the main ingredient in sorbet. In cooking, it can be used in sauces, marinades, and as thickener and flavoring in soup and sauces as substitute for milk (Scarpa 1997). Buttermilk is also used in preparation of waffles as well as *blintzes* (Horn et al. 1997).

Sour Cream

Sour cream is a thick, viscous fermented dairy product with a white, rather shiny appearance. It is a popular ingredient that is mainly consumed with warm or hot foods such as baked potatoes or Mexican foods such as burritos. Due to its special characteristics sour cream can remain stable even when in contact with hot foods. This specific characteristic is obtained through the use of a certain bacterial strain that is used in

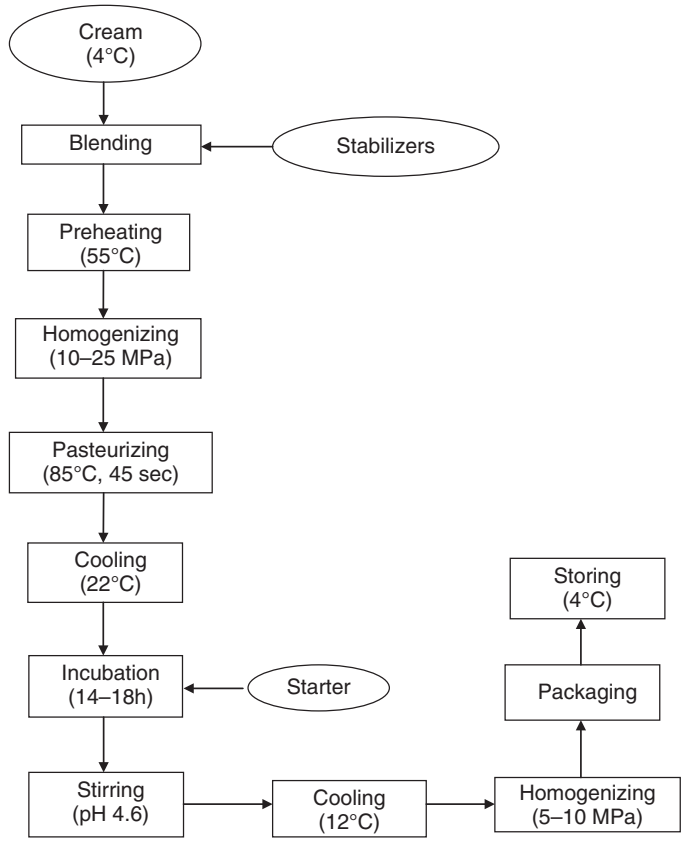

Figure 13.3. Flow process chart for typical sour cream manufacture. Adapted from Meunier-Goddik et al. (2004).

fermentation of raw dairy ingredients. Various sour cream products are available in the market. Unlike yogurts and cheeses, the variation in sour cream is mainly based on the fat content and other non-dairy ingredients that are added.

By definition, sour cream is a product of pasteurized cream that is soured by lactic-acid-producing bacteria; it should contain not less than 18% milk fat and not less than 0.5% titratable acidity as lactic acid (FDA 2008). Other dairy or non-dairy ingredients can be added; however, the fat content of the final product should not be less than 14.4%. At present, low-fat and nonfat sour cream also are available.

Sour Cream Characteristics

As the name implies, sour cream is characterized by its sour taste due to the accumulation of lactic acid from LAB fermentation of lactose. However, light- and mild-flavored sour cream is also widely available in most supermarkets. Sour cream exhibit diacetyl flavor as well as that from acetic acid, acetaldehyde, and dimethyl sulfide (Meunier-Goddik 2004).

Sour Cream Formulation

Of notable importance is the starter culture used to ferment the milk cream. The starter culture used in sour cream preparation con-

tains both acid-producing and flavor-producing bacteria (Folkenberg and Skriver 2001), and to a lesser extent a small amount of rennet (Kosikowski and Mistry 1997).

The acid-producing bacteria commonly used include *Lc. lactis* subsp. *lactic* and *Lc. lactis* subsp. *cremoris*; the common flavor-producing bacteria are *Lc. lactis* subsp. *lactis* biovar *diacetylactis* (or Cit^+ Lactococci) and *Leuc. mesenteroides* subsp. *cremoris.* A small amount of rennet gives sour cream its heavy body. The viscosity of sour cream is usually altered by an addition of milk powder (Kosikowski and Mistry 1997). Exopolysaccharide-lactic-acid-producing bacteria and low-fat cream (6% fat) also have been used (Adapa and Schmidt 1998).

Manufacture of Sour Cream

Sour cream manufacture consists of several key steps such as mixing the milk cream with stabilizer, homogenization, pasteurization, cooling to 22°C (72°F), mixing in the culture, fermentation, breaking of the coagulum, homogenization, and storage (Figure 13.3). The ingredients can be added directly into standardized cream or before it is standardized when dry ingredients are used. The ingredients are then mixed, preheated, and homogenized before cooling to 22°C (72°F). Starter cultures are then mixed in and the mix is fermented at 22°C (72°F) for 14 to 18 hours. The sour cream is further cooled to 12°C (54°F), homogenized, packed, and stored at refrigerated temperature (Meunier-Goddik 2004).

Physicochemical Changes During Fermentation

The addition of two different cultures in milk cream fermentation lead to the production of various lactose derivatives through two different pathways. The acid-producing cultures follow the homofermentative pathway, mainly produce L-lactate from sugars (lactose), and grow at around 10ºC (50ºF). The lactic-acid produced by the acid-producing bacteria is around 0.8% depending on the strain used (Cogan 1995). The production of lactic acid decreases the pH of the fermented cream and provides the characteristic mild, sour taste.

The flavor-producing culture converts lactose into D-lactate, ethanol, and carbon dioxide through the heterofermentative pathway. Acetate also can be produced when external electron acceptors such as O_2 and citrate are available. The citrate content of milk is around 8 mM and varies throughout lactation. Citrate metabolism is an important property of some mesophilic cultures such as *Lc. lactis* subsp. *lactis* biovar *diacetylactis.* This metabolism produces acetate, diacetyl, acetoin, 2,3-butanediol, and carbon dioxide. Diacetyl is one of the major flavor compounds responsible for the typical flavor of cultured buttermilk, ripened cream butter, sour cream, fromage frais, quark, and cheddar cheese. Acetate also contributes to the flavor, but acetoin (a derivative of diacetyl) and 2,3-butanediol do not (Monnet et al. 1995).

The changes during milk cream fermentation also include an increase in viscosity as a result of lowering the pH and production of other metabolites by the bacterial culture. This increase is due to disaggregation of calcium submicelles as the colloidal calcium phosphate solubilizes and aggregates into a more ordered system (Fox et al. 2000). The production of other metabolites such as exopolysaccharide also alter the viscosity. This polysaccharide contains galactose, glucose, fructose, mannose, and other sugars, depending on strain and growth conditions (Nakajima et al. 1990, Cerning et al. 1992, Tamime and Robinson 2007).

Quality of Sour Cream

The quality of sour cream depends on the quality of the raw material and the manufacturing conditions, and can be grouped into

flavor and texture parameters. Sour cream is highly vulnerable to lipid-associated off flavors such as rancidity and oxidized flavor. Other off-flavors such as lack of fine flavor, lack of cultured flavor, high acid, and bitterness are common quality concerns (Meunier-Goddik 2004).

The rancid flavor of sour cream is due to lipolysis of fatty acid by lipase that originates from the milk itself or from the culture. Lipolysis only occurs if the milk fat globule breaks and the lipase can gain access to the milk fat. This can be avoided by pasteurizing the milk before or immediately after homogenization to deactivate most of the enzymes in the raw material. The oxidized flavor, also known as metallic, comes from oxidation of milk fat and phospholipids that are catalyzed by divalent cations such as iron and copper. Therefore, avoiding contact of the raw material with these metals, such as in parts of pipes, protects the product from developing this flavor (Meunier-Goddik 2004).

The choice of starter culture can affect the cultured flavor and acidity of sour cream. Lack of flavor or high acid renders the product unpleasant. These problems can be solved by changing the culture composition (Meunier-Goddik 2004). Bitterness is due to the activity of proteolytic enzymes in the raw material. The bitter taste may develop during storage. The choice of good-quality raw material and a higher pasteurization temperature can limit the possibility of sour cream developing a bitter taste.

Texture is an important characteristic of sour cream. It is usually served with warm foods; therefore, it must remain highly viscous when in contact the surface of warm foods. This textural parameter is a function of the cheese pH. High pH increases meltability, decreases firmness, and causes a swollen structure (Monteiro et al. 2009). Incorporation of proper stabilizers is important to produce sour cream that will not cling to the spoon (too firm) or melt when in contact with a hot food surface (too weak). The proper choice of stabilizers and processing procedures also prevents a grainy product as well as whey syneresis (Meunier-Goddik 2004).

Shelf Life of Sour Cream

The shelf life of sour cream under refrigerated temperature is about six weeks (Warren 1987) or roughly 25 to 45 days (Meunier-Goddik 2004). The shelf life is determined mainly by the quality of the milk used to produce the milk cream and its pretreatments. Quality attributes often decrease as a sour odor and bitter taste increases.

Use of Sour Cream

Sour cream is commonly added to baked potatoes and Mexican foods such as burritos. Its heat-stable characteristics make it a highly suitable ingredient for such foods. The flavor attributes of sour cream remain appreciable when served with food such as baked potatoes, ham, and pineapple, or baked potatoes with chili corn carne. However, in flavor-rich foods such as Mexican foods, the mild flavor of sour cream can easily be masked; hence it is of little significance.

Sour cream also is used in combination with cottage cheese and cream cheese in preparation of cheesecake such as New York-Style cheesecake. While most cheesecakes are served as cold food, this cheesecake is usually served as it comes out of oven with a coat of sour cream (Horn et al. 1997); hence, the importance of the heat-stable texture of sour cream.

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Chapter 14

Functional Ingredients from Dairy Fermentations

Ebenezer R. Vedamuthu

Introduction

Present-day consumers are health-conscious and well-informed about the link between food and health. This has led to regulations requiring that packaged foods carry nutrient and ingredient labeling. In nutrient labeling, the composition and relative amounts of individual nutritive components must be listed for an average serving of the food. In ingredient labeling, all of the ingredients contained in the food must be declared. Modern food marketing involves shipment of food across state lines and national boundaries. Often, the time lapse between production and consumption of packaged food is fairly long, which has spawned various processing and preservative and protective technologies. These technologies aim to deliver processed food that retains most if not all of the attributes with regard to body, texture, and flavor of the food in its fresh form. An additional concern is ensuring the safety and wholesomeness of the food. These demands have called for innovative technologies in processing, preservation and protection.

In the modern competitive marketplace, the food industry strives to introduce novel forms, textures, and flavors (or a combination of flavors) in packaged foods to meet consumer demands and a desire for variety in foods. These demands pose challenges in maintaining to the best extent possible the physico-chemical properties of the original foods and ensuring their safety as formulated foods. To meet these requirements, the industry uses chemical stabilizers, emulsifiers, antioxidants, sweeteners, flavoring ingredients, and other chemical compounds to preserve freshness and protect the food from chemical and microbial deterioration as well as the development of pathogenic agents and their toxins. All these ingredients must be declared on the label.

In the European Union, many of these chemical ingredients and other additives have been assigned EU numbers, which also must be listed. Consumers find it daunting to comprehend the purpose and need for these additives. Furthermore, the public is bombarded with a cacophony of voices that raise their anxiety about the health risks of consuming the long and bewildering list of unfamiliar chemicals found on the food labels. Frye and Kilara (2008) have succinctly summarized the regulations for product standards and labeling for dairy products. Further information pertaining to regulations and labeling guidelines are summarized by Patel et al. (2008).

To assuage consumer anxiety and win their confidence, the industry strives to develop technologies to allow clean labels. To achieve this, chemical additives are avoided to the extent possible. To replace the chemicals, suitable natural or biologically derived, food-grade alternatives are employed. Biopreservatives fill this niche, aiding in protecting and

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preserving foods against spoilage and helping to prevent food-borne transmission of pathogens and their toxins. Functional bioingredients fulfill other functionalities of chemicals used for various purposes listed earlier. This chapter considers bio-ingredients developed *in situ* during the manufacture of dairy products as well as those derived in separate fermentations and the resultant ingredient(s) added to the dairy product to obtain the desired functionality.

Functional Bio-ingredients: Live Cultures

Use of Live Functional Cultures

Functional cultures are used to develop *in situ* the desired body, texture, and flavor attributes in dairy and other foods. A very good example of functional cultures used in dairy fermentations is the inclusion of exopolysaccharide-producing (EPS) starter cultures.

One of the traditional uses of EPS cultures is found in viili production. The desired heavy, somewhat slimy body, and the ropy, stringy texture of viili are imparted by the EPS generated by the starter lactococci in viili starters. Vedamuthu (2006) and Robinson et al. (2002) have described the attributes and production of viili.

During the past decade, selected EPS-producing lactococci have been used because of the escalating cost of nonfat milk solids used in fortifying fluid skim milk for cultured buttermilk, and for increasing the solids level in cultured sour cream. Such cultures are called heavy body cultures. The EPS is generated *in situ* during the respective fermentation to impart a heavy body without sliminess or ropiness under normal production conditions of these products. The use of such heavy body cultures saves money by helping to decrease or even completely replace the costly nonfat dry milk solids needed. The smooth texture and heavy body imparted by EPS-generating lactococci also suppress foaming during cultured buttermilk packaging operations, which formerly caused problems in completely filling the bottles.

Exopolysaccharide-producing lactococci are also susceptible to lysis by virulent phages. To overcome phage-related failures in buttermilk and sour cream manufacture, rotation of starter cultures containing EPS-producing strains of variable phage susceptibility is necessary. Unfortunately, the availability of a large number of EPS-producing lactococci is limited. Procedures for converting non-EPS lactococcal strains to EPS-generating variants by conjugative transfer of plasmid coding for EPS production have been developed and patented (Vedamuthu, 1989). This technique has provided a means for developing sufficient numbers of heavy body cultures with EPS-generating lactococci of variable phage-sensitivities.

Nielson (1975) listed the following attributes for a high-quality yogurt with respect to the body characteristics. The body of yogurt should have a relatively high viscosity and should be firm and cohesive enough to be removed from the container and eaten with a spoon. It should have enough resilience to withstand normal handling during post-incubation operations and by consumers in the home without undue wheying off or shattering. The solids content of the yogurt mix should be high to obtain the smooth, viscous body resistant to easy shattering or wheying-off.

Stabilizers are used to further enhance the water-holding capacity of yogurt and increase its viscosity and smoothness. Common yogurt stabilizers include gelatin, alginates, carrageenan, carob gum, guar gum, starch, and carboxymethyl cellulose. Sometimes multiple stabilizers are used in combination. Stabilizers should always be declared in the label. Kosher requirements do not allow the use of gelatin, and Kosher-grade gelatin is very expensive.

Because of these drawbacks, namely the cost factor and the need to declare stabilizers on the ingredient label, the industry prefers to use yogurt starters that contain EPS-producing strains. Exopolysaccharide-generating strains are known among *Streptococcus thermophilus* (coccus) and *Lactobacillus delbrueckii* subspecies *bulgaricus* (rod). Starters containing either EPS-generating coccus or rod or a combination of both are available. The EPS generated during fermentation by the starters provides the functionality required for the body and textural attributes desired in the finished yogurt. Furthermore, the smooth, viscous, and heavy body produced by EPS-generating starters also aids in the uniform suspension of fruit in fruit-flavored yogurts.

EPS-generating rod-coccus cultures also are used to produce yogurt drinks, which are now popular in the United States and Europe. The important characteristic of yogurt drinks is the thick, smooth, silky body and texture that resembles a milkshake. Yogurt starters containing EPS-producing rod-coccus components yield a product with a thick, heavy, viscous body, which, when stirred gently through the use of slow speed agitators, imparts a milkshake-like consistency. For large-scale industrial production of yogurt drinks, gentle handling, agitation, and pumping is difficult to achieve. Therefore, a suitable combination of an EPS-producing culture and a stabilizer system is necessary. The use of such EPS-producing cultures in applications such as yogurt drinks has been patented (Vedamuthu, 1982).

EPS-producing cultures also have been used to improve cheese quality. Researchers at Utah State University observed that a capsule-producing (Cps^+) *Streptococcus thermophilus* strain (MR-1C) could increase moisture content and functional properties of low-fat mozzarella cheese (Perry et.al., 1997; Low et.al., 1998). They classified EPS produced by lactic acid bacteria (LAB) into two groups: capsular (Cps), which is tightly associated with the cell wall of bacteria, and loose slime, which is secreted and non-cell associated, designated as EPS. In a later study, the Utah State University group (Peterson et al., 2000) compared the effect of *Streptococcus thermophilus* (ST) strains producing Cps and EPS, respectively, on low-fat mozzarella cheese functionality and cheese whey viscosity. They used four ST strains, each paired with *Lactobacillus helveticus* LH 100 (a commercial mozzarella cheese starter strain) to make low-fat mozzarella cheese.

The starter pairings were as follows:

Pairing A: LH 100 and ST strain MR-1C (capsular exopolysaccharide producer Cps^+)

Pairing B: LH 100 and ST strain DM 10 (a non-capsular mutant of MR-1C, Cps^-)

Pairing C: LH 100 and ST strain MTC 360 (a loose slime producer, EPS^+)

Pairing D: LH 100 and ST strain TAO61 (a commercial strain that did not produce either type of exopolysaccharide)

The cheeses were analyzed for chemical composition, starter bacterial counts, and cheese melt properties, and the cheese yields were determined. The results revealed that there were significant differences in the moisture and protein contents between the cheeses. Hence, the cheese yields also varied. There were no significant differences in other chemical indices measured, namely fat content and calcium and sodium chloride levels. The pH of the cheeses did not vary significantly.

The moisture levels, from highest to lowest, were as follows: Pairing C (57%), Pairing A (53%), Pairing D (51%), and Pairing B (49%). The protein contents were inversely correlated with the moisture levels: (Pairing B, 26%; Pairing D, 25%; Pairing A, 24%; and Pairing C, 22%). The cheese yields were directly correlated with the moisture contents; cheeses made with pairings C and A had greater yields than those made with pairings B and D.

The starter populations among cheeses were not significantly different. The test for cheese melting functionality showed that the cheeses made with Pairing C displayed the highest melt distance (12.5 cm) vs. pairings A (10.4 cm), D (9.3 cm), and B (8.8 cm). From these observations it was concluded that the exopolysaccharide-producing strains improved the melting functionality of the cheese, as well as the yields. However, the researchers noted that the cheese made with the Pairing C (containing the EPS^+) strain was sticky, soft, and difficult to shred.

The whey from cheese made with Pairing C was viscous and difficult to process through ultrafiltration equipment. On the other hand, the cheese of Pairing A, containing the Cps^+ strain, did not have these drawbacks. Hence, strain MR-1C (Cps^+) was recommended for use in producing low-fat mozzarella cheese with good melting functionality, higher yields, and whey with good downstream processing qualities. ST strain MR-1C is commercially available for cheese manufacturers.

Hassan et al. (2004) reported on the favorable modification of body and texture of Karish cheese through the use of EPS-producing LAB starter, in this case *Lactobacillus delbrueckii* ssp. *bulgaricus* CHCC 769. It was paired with ST strain CHCC 3534. Karish cheese is a soft Egyptian cheese made from skim milk and acid coagulation. Due to the lack of fat and high density of the protein matrix, the cheese has a dense, firm, brick-like texture that is not cherished by consumers. Hassan et al. (2004) made Karish cheese using a non-exopolysaccharide-producing combination consisting of ST 5843 and *Lactobacillus delbrueckii* ssp. *bulgaricus* (a non-EPS mutant of CHCC 769) in one vat. Cheese was made in a parallel vat with inoculated CHCC 769/ST strain CHCC 3534. The cheeses were analyzed for moisture, microstructure by cryoscanning electron microscopy, and rheological properties. The overall data showed that the body and texture of Karish cheese could be favorably modified through the use of EPS-producing strains in starter cultures. Hassan (2008) has extensively reviewed the possibilities and challenges of applying exopolysaccharide-producing LAB in dairy foods.

Flavor enhancement of cottage cheese is another area in which cultures are used to improve quality. In the United States, a fairly high level of diacetyl is desired in the cream dressing of cottage cheese. Babel and Mather (1961) described a process using *Leuconostoc mesenteroides* ssp. *cremoris* to boost the diacetyl content in cream dressing for cottage cheese: A mixture of skim milk and cream is heat-treated, cooled to 21°C to 25°C (69.8°F to 77°F), and inoculated with 1% to 2% of a culture of *Leuconostoc mesenteroides* ssp. *cremoris* (strain 91414). The inoculated mixture is incubated at the same temperature range for 24 to 48 hours to sufficiently increase the numbers of the inoculum. At the end of incubation, the pH of the mixture is adjusted to about 4.3 with sterile citric acid. The lowered pH value promotes diacetyl synthesis by enabling the rapid uptake of citrate. The acidulant used to adjust the pH is also the precursor for diacetyl. Furthermore, the pH is conducive for the diacetyl synthesis to proceed optimally. To promote diacetyl synthesis, the pH adjusted-mixture is further incubated at 21°C to 25° C (69.8°F to 77°F)for 24 hours, and immediately chilled. The chilling arrests the reduction of diacetyl to not-so-flavorful acetoin. The chilled mixture is ready for application on dry cottage cheese curd. A patent based on this procedure was awarded to Babel and Mather (1961), and the process has been in use commercially for many years.

Gilliland et al. (1970) described the production of concentrated cultures of *Leuconostoc citrovorum,* which may be supplied to processors in frozen form. The use of concentrated culture obviates the need for the initial 24 to 48 hours of incubation of the creaming mixture to build up the flavor bacterial population. The measured dosage of the

concentrated culture may be added directly to the creaming mixture, adjusted to pH 4.3 with citric acid, and the process continued as described by Babel and Mather (1961).

One of the earliest applications of separate flavor bacteria (not part of the starter culture) in cultured dairy products was promoted by Lundstedt (1962a,b). In the first paper, Lundstedt (1962a) described a medium consisting of citrated cottage cheese whey and pancreatic extract (0.2%) that was found to support the growth of both *Leuconostoc citrovorum* and Cit^+ *Lactococcus lactis* ssp. *lactis* (also referred to in the literature as *Lactococcus lactis* ssp. *lactis* biovar *diacetylactis*; hitherto abbreviated here as SD) to high cell numbers. In the second paper, Lundstedt (1962b), added 0.4% of a citrated whey culture of SD, which had been grown for 18 hours at 21°C (69.8°F) to 224 g of creamed cottage cheese curd. The cheese was then held at 3.3°C (37.9°F) for 3 days and examined. Within 3 days of refrigerated holding, the cheese had developed a "typical well-rounded butter aroma and very pleasant flavor." He also monitored the diacetyl concentrations in cottage cheese, prepared as described earlier, over 18 days of holding at refrigerator temperatures. The SD-inoculated cheese exhibited progressive build-up of diacetyl concentration up to 10 days, beyond which there was no change in the diacetyl concentration up to 18 days. He found a similar trend with *Leuconostoc citravorum,* but the level of diacetyl obtained was lower than was achieved with SD culture (strain 26-2). This was the first quantitative demonstration of build-up of diacetyl concentrations at refrigeration temperatures, and it laid the foundation of further technological developments in this area.

In the same paper, Lundstedt (1962b) demonstrated the production of a concentrated slurry of SD cells by back flushing on a Seitz filter. The use of such concentrated cells to inoculate chilled, creamed cottage cheese curd showed considerable improvement in the sensory properties of the cheese due to elevated diacetyl concentrations. Based on these studies, Lundstedt (1962) patented the process for application in creaming the mixture for cottage cheese and for boosting the diacetyl flavor in cream and Neufchatel cheeses and butter and margarine.

Later technological advances led to the use of centrifuges (de-sludge type centrifuges) to facilitate the production of highly concentrated bacterial cultures. With this advancement frozen concentrated cultures could be thawed and immediately added to the chilled creaming mixture for dressing the chilled cottage cheese curd, with the flavor and aroma developing during the refrigerated holding of the packaged cottage cheese. The first patent for an application using a highly concentrated culture of SD was issued to Sing (1976). The process is widely used by the dairy industry in the United States.

Live Probiotic Cultures

Although probiotic cultures may be considered as functional cultures, they are treated separately here because their functionality is not related to imparting quality attributes such as body, texture, and flavor. The functionality of probiotic cultures relates to adding value to dairy products and imparting health attributes. The dairy product merely serves as a vehicle for the delivery of the desirable health-imparting cultures. According to the United Nations' Food and Agriculture Organization, "probiotics are live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host." (Shelke, 2003).

There are many benefits of regular intake of probiotics, including alleviating lactose maldigestion; reducing serum cholesterol; stimulating immunity; providing antimicrobial, antimutagenic, and anticarcinogenic effects; and maintaining intestinal health and general well being (Shah, 2000). Other authors have listed additional benefits

(Sanders and Huis in't Veld, 1999; Chandan, 1999). The evidence equivocally supports the maintenance of intestinal health as a benefit. The most widely heralded probiotic species belong to the genera *Lactobacillus* and *Bifidobacterium*. These species are normal inhabitants of the human gut and have been shown to play a regulatory role in its ecology and microbial flora (Chandan, 1999; Sanders and Huis in't Veld, 1999). Selection criteria for probiotics reported by various authors were summarized by Shah (2006).

Sweet acidophilus milk is the forerunner of the present-day probiotic milks. It is made by adding a sufficient quantity of frozen concentrated culture of an authentic *Lactobacillus acidophilus* strain (preferably of human origin, bile-resistant, technologically adaptable for propagation to high numbers, concentration by either centrifugation or ultrafiltration, preservation by freezing or lyophilization, and capable of maintaining viability in cold pasteurized milk or skim milk over three weeks of refrigerated holding) to cold, freshly pasteurized milk. After mixing, this provides a viable cell count of 2 million to 4 million colony-forming units/ml. The seeded milk is packaged for marketing. The history and development of sweet acidophilus milk is discussed by Vedamuthu (2006).

The early version of probiotic milk contained a mixture of *Lactobacillus acidophilus* and strain(s) of *Bifidobacterium* sp., and was called A/B milk. Later, branded products containing proprietary strains of *Lactobacillus* spp. and *Bifidobacterium* spp. were selected on the bases of purported, proven probiotic benefits. Extensive discussion of the history and current status of probiotic dairy products may be gleaned from recent reviews (Chandan, 1999; Shah, 2006; and Vedamuthu 2006). Table 14.1 lists some of the well-known proprietary probiotic bacteria used in dairy products.

Table 14.1. Probiotic bacterial strains marketed by leading dairy food manufacturers and dairy starter suppliers.

Manufacturer/ Supplier	Probiotic strains
Chr. Hansen	*Lb. acidophilus* LA1/LA5 *Lb. bulgaricus* Lb 12 *Lb. paracasei* CRL 431 *Bif. animalis* Bb 12
Danisco	*Lb. acidophilus* NCFM® *Lb. acidophilus* La14 *Lb. casei* Lpc 37 *Bif. lactis* B 104
Nestle	*Lb. johnsonii* La 1
Yakult	*Lb. casei* Shirota *Bif. breve* (Yakult)

Of the various food products that have been examined, dairy products appear to be the most suitable for delivering probiotic cultures. Yogurt, which is very popular among adults and children, has become the most widely used vehicle for delivering probiotics. Yogurts containing probiotic cultures are currently the norm on supermarket shelves. Different proprietary probiotic strains belonging to the genus *Lactobacillus* are widely used in addition to yogurt starter bacteria (*Streptococcus thermophilus,* ST, and *Lactobacillus delbrueckii* subsp. *Bulgaricus,* LB). These strains often are used in combination with *Bifidobacterium* spp. in plain and flavored yogurts.

The probiotic cultures are declared on the product labels. Appropriate health claims for the declared probiotic strains are often listed on the packages of specific yogurts.

The probiotic strains are added along with starter bacteria before the commencement of the fermentation, or, in most cases, they are added at the post-fermentation stage; they are then mixed in and packaged. Because of the inability of many of the probiotic strains to compete with the yogurt starter bacteria, and in some cases to avoid the undesirable acetic acid flavor that is generated by bifidobacteria, it is desirable to add the probiotic strains at the post-fermentation stage.

Adding probiotic strains after fermentation is desirable because the probiotic benefit supposedly depends upon the viable numbers of the probiotic strains at the time of consumption by the consumers. The inability of probiotic *Lactobacillus acidophilus* and bifidobacterial strains to compete with the yogurt starter bacteria during fermentation, coupled with the suppressive effect of hydrogen peroxide generated by LB, makes it difficult to meet the arbitrary standards for viable counts for probitics that are generally accepted by the yogurt industry. The probiotic yogurt industry is self-regulated, because there are no official regulatory requirements. Further, there are no recognized accurate standard methods for the differential enumeration of probiotic strains in the presence of yogurt starter bacteria. Until this shortcoming is resolved, standards for probiotic yogurt is unforeseeable.

Recently, Grattepanche et al. (2008) have presented the pros and cons of cheese as a vehicle for delivering probiotic bacteria. Champagne et al. (2005) reported that probiotic cultures consisting of *Lb. acidophilus* and bifidobacteria are mostly added to milk, yogurt, ice cream, and desserts. However, the conditions encountered in these products are very different from those occurring in the natural habitat of probiotic bacteria, namely, the gastrointestinal tract of humans. The product composition and the microecological environment could have deleterious effects on the viability of probiotic strains, which is considered to be the most important prerequisite for beneficial health effects of probiotics.

Roy (2005) has suggested that a minimum intake of 10^8 to 10^9 viable cells of probiotics is necessary to provide a therapeutic effect. Boylston et al. (2004) argued that because of its relatively limited acidity, low oxygen level, high lipid content (which provides protection), and low storage temperatures, cheese is a suitable carrier for delivering live probiotic bacteria. Furthermore, the cheese core could be considered an anaerobic environment with a very low redox potential (E_h) of about −250 mv (Beresford et. al., 2001), which is favorable for the survival of probiotic bacteria. Grattepanche et al. (2008) have summarized the data from various studies using a variety of cheeses, and shown that cheese indeed could serve as a good carrier to deliver high numbers of viable probiotic cultures. The authors, however, caution that incorporation of probiotics in cheese could lead to compositional, body, texture, and flavor deviations in cheese. They suggest that careful probiotic strain selection and process adjustments are necessary to overcome such cheese quality problems. They stress the need for further research in these areas.

Biopreservatives: Live Protective Cultures

The term protective cultures can be applied to traditional starter and associated bacteria used in the production of fermented dairy products to protect them from spoilage and pathogenic and enterotoxigenic bacteria by their suppressive or inhibitory activity. Fortuitously, the flavor bacteria that are added to cottage cheese to enhance desirable dairy notes such as diacetyl flavor were found to exert a suppressive effect on spoilage and pathogenic and enterotoxigenic flora often encountered in this product. The inhibitory properties of live cells of citrate fermenting *Lactococcus lactis* subsp. *lactis* (also referred to as biovar *diacetylactis*) were reported by Vedamuthu et al. (1966) to inhibit Gram-negative spoilage bacteria in creamed cottage cheese, as well as enteropathogenic Gram-negative bacteria such as *Salmonella* spp. The inhibitory effect of the same bacterium on spoilage and pathogenic bacteria in milk and broth systems was reported by Daly et al. (1970a,b; 1972) and on *Psueudomonas* spp. by Pinheiro and Parmalee (1968).

Branen et al. (1976) described the purification of antimicrobial substances active against *Pseudomonas* spp., elaborated by *Leuconostoc mesenteroides* ssp. *cremoris* and citrate-metabolizing *Lactococcus lactis* subsp. *lactis* strains.

The repeated observations by various researchers on the inhibitory effect of the citrate-metabolizing *Lactococcus* subspecies and its flavor-generating properties culminated in the filing of patents by Sing (1976) and Gonzalez (1984, 1986). The antimicrobial activity of *Leuconostoc mesenteroides* ssp. *cremoris* also was noted by several investigators (Mather and Babel, 1959; Marth and Hussong, 1963; and Sorrels and Speck, 1970). Based on these findings, Babel and Mather (1961) patented the application of *Leuconostoc mesenteroides* ssp. *cremoris* in cream dressing for cottage cheese. Both the citrate-fermenting *Lactococcus lactis* ssp. *lactis* and *Leuconostoc mesenteroides* ssp. *cremoris* are extensively used (as chill additives as discussed in the previous section) by the industry to control unwanted spoilage and pathogenic/enterotoxigenic bacteria in creamed cottage cheese. In addition, these organisms generate desirable diacetyl flavor during its initial holding in the cooler and its distribution through the marketing channels.

The patents filed by Gonzalez (1984, 1986) on the use of lactose-negative and citrate-permease-negative *Lactococcus lactis* subsp. *lactis* (biovar *diactylactis*) for chill addition to extend the shelf-life were uniquely designed for the cottage cheese market in California, where high acidity and high diacetyl flavor did not find favor with the customers. In addition to its application in cottage cheese in the United States, Cit+ *Lactococcus lactis* subsp. *lactis* was found to be inhibitory to *Salmonella* in broth, milk, and cottage cheese systems (Vedamuthu et al., 1966). Based on these observations, currently, frozen concentrated cultures of this bacterium are widely used in Europe as a direct-to-vat adjunct inocula to control *Salmonella* in French surface-ripened semi-soft cheese made from raw milk.

Recently, a trade release entitled "Protective Cultures" by Danisco (2005b) defined the appellation as follows: "Protective cultures are bacteria especially selected and developed for their ability to control the growth of pathogenic and/or spoilage microorganisms in fermented foods." The release deals with protective cultures for both fermented dairy products as well as meat products. The bacteria identified for this function are strains of *Lactobacillus plantarum*, *Lactobacillus rhamnosus, Lactobacillus sakei, Lactobacillus paracasei,* and *Propionibacterium freudenreichii* subsp. *shermanii.* With the exception of *Lactobacillus sakei*, all of the other species are associated with fermented dairy products. *Lactobacillus sakei* is more frequently encountered in fermented meats, and is not reported to occur in fermented dairy products.

Single cultures or combinations of these species are sold for various applications under the trade name HOLDBAC™. Specific cultures in this series are recommended for specific functions in different fermented dairy products. The release recommends the use of HOLDBAC™ for growth control of *Listeria* in soft and smear cheese. HOLDBAC™ LC is recommended for the growth control of undesirable *Leuconostoc*, enterococci, and heterofermentative lactobacilli in semi-hard and hard cheeses. Leuconostocs are often implicated in late gas splits in semi-hard cheese, and heterofermentative lactobacilli also contribute to gas openings and certain flavor defects such as fermented flavors or fruitiness. For growth control of yeasts and molds in fresh fermented dairy products such as yogurt, sour cream, quark, cottage cheese etc., HOLDBAC™ YM-B and YM-C are recommended. Grattepanche et. al (2008), on the basis of published reports, mention that HOLDBAC™ YM-B consists of *Propionibacterium freudenreichi* subsp.

shermanii JS and *Lactobacillus rhamnosus* LC705. The YM-C culture is comprised of *Propionibacterium freudenreichii* subsp. *shermanii* JS and *Lactobacillus paracasei* SM20. They have also listed the possible metabolites from these bacteria that could be attributed to their respective suppressive effect on the specific undesirable spoilage flora.

It is probable that the *Listeria* growth control (as claimed for HOLDBAC™) is achieved by *Lactobacillus plantarum*. Among the various bacteriocins produced by lactic acid bacteria (LAB), pediocins elaborated by *Pediococcus acidilactici* is by far the most effective against *Listeria monocytogenes*. Ennahar et al. (1996) reported the isolation of a strain of *Lactobacillus plantarum,* designated WHE 92, that produced an identical bacteriocin (the primary structure was identical to pediocin AcH) that was equally inhibitory to *Listeria.* The *Lactobacillus* strain was isolated from Muenster, a smear-surface soft cheese. The unique feature in the production of the pediocin derived from the *Lactobacillus* strain related to its undiminished elaboration, even at pH values as high as 6.0. Ennahar et al. (1996) suggested that the ability of *Lactobacillus plantarum* WHE 92 to thrive in cheese systems and its undiminished elaboration of pediocin at pH values above 5.0 make it attractive for application in dairy products such as cheeses, where the pH is above 5.0 in most varieties.

Danisco (2005b) listed several benefits that could accrue through the use of HOLDBAC™ culture series: *Listeria* reduction and food safety improvement, shelf-life extension or maintenance (of quality) by avoiding microbial organoleptic degradation, reduction of supply chain and distribution costs, replacement of chemical preservatives by natural and safe solution (allowing clean labeling), and allowing for new product formulation. Additionally, these cultures are compatible with normal starter cultures used in the production of these dairy products. Another such product on the market is FARGO® 25 (Kerry, 2004a).

Functional Bio-ingredients: Microbial Metabolites

The following section discusses microbial metabolites derived through fermentations that may or may not contain live residual microbial cells (used in fermentation), either in liquid form or as dried powders. Essentially, these bioingredients consist of crude fermentates that have functional applications. They include components of the fermentation media, microbial metabolites, dead or injured or residual live cells used in the fermentation, and if dried, some approved processing aids.

Bio-thickeners

Bio-thickeners include fermentation-derived gums and exopolysaccharides (EPS) that could be used to increase the body, viscosity, water-holding capacity, and resilience to mechanical shear and other processing operations. Thickeners derived from fermentation may be purified compounds or crude EPS occurring as a part of the fermentate. Dextran and xanthan gum are examples of purified compounds. In this discussion, thickeners derived from fermentations using safe microorganisms traditionally associated with food fermentations are considered.

The production of dextran by *Leuconostoc mesenteroids* subsp. *dextranicum* from sucrose is well known. However, the application of dextran derived from the *Leuconostoc* subspecies as a thickener or texturizer in dairy products is a relatively recent development. Pucci and Kunka (1990) described a patented process for the production of a unique dextran produced by *Leuconostoc mesenteroides* subsp. *dextranicum* NRRL-B18242 in a milk substrate containing sucrose. The milk culture containing dextran is dried to a powder, which is suitable for thickening and texturizing cultured milks and

flavored milks and may be used as a stabilizer in ice cream. When grown in a sucrose-salt medium, the organism produces a slushy, applesauce-like particulate, a gel-like structured dextran product that exhibits antimicrobial properties and is stable at room temperature for 12 months. The dextran from this strain was examined for polymer composition and degree of branching by C-NMR analysis. It was found to contain a mixture of polymers containing 1, 6-glucopyranose with branching approximately every 11 residues as well as 2% fructofuranose. This process for producing dextran has been commercialized.

The trade literature for these products claims that they fulfill the requirements for all natural ingredient declaration and comply with 21 CFR 131.146 for dried cultured skim milk. Furthermore, these products provide good textural properties and an improved creamy, buttery mouth feel in ice cream and other dairy products. Among the additional advantages, these products could reduce or replace non-fat milk and sweetener within the fat content limits in dairy formulations, provide heat shock protection in ice cream, and allow higher aeration compared to products without emulsifiers or gums (Kerry, 2004b).

Bio-flavorings

One of the earliest applications of bioingredients in dairy products was the development of flavor enhancers for creamery butter. Culturing cream for butter making has not been widely practiced because of the greater propensity of salted, cultured butter to undergo fat oxidation and other chemical deterioration during holding than sweet cream butter. Sweet cream butter, however, lacks good "buttery" flavor, and is often criticized as flat and lacking in flavor. To remedy the lack of flavor, procedures have been developed to add cultured flavor directly to butter. After churning and working of butter, measured amounts of sweet cream buttermilk (drained from the churn) cultured with *Leuconostoc* strains are worked into the butter at the time of moisture adjustment, to adjust the moisture content to the legal limit (Vedamuthu, 1994). A suitable procedure for these operations was described by Seas et al. (1960).

Lundstedt (1962) patented a procedure in which citrated whey (preferably cottage cheese whey) is suitably "sterilized" and then inoculated with a strain of *Streptococcus diacetilactis* (cit$^+$ *Lactococcus lactis* ssp. *lactis*) and cultured at 21°C (69.8°F) for 18 hours and then added to butter "at any stage before working or texturizing, provided it is not subjected to temperatures in excess of about 50°C (122°F)." During the holding of the butter in cold storage, diacetyl levels gradually increase to yield a fine flavored butter. For additional details, refer to the patent (Lundstedt, 1962).

Hugenholtz (1993) described a high-diacetyl-producing starter, designated NIZO 4/25, from which a *Lactococcus lacis* strain that accumulated very high concentrations of α-acetolactate from citrate was isolated. This strain had multiple designations, including Ru4, SD806, and 425A. Biochemical characterization of the strain revealed that it lacked α-acetolactate decarboxlase, which resulted in the accumulation of α-acetolactate. α-acetolactate is the precursor of diacetyl and is an unstable compound that can be chemically converted to diacetyl. Because of the accumulation of α-acetolactate, this strain produces high amounts of diacetyl when used in fermented dairy products.

Exploiting the metabolic quirk in strain NIZO 4/25, Veringa et al. (1974) developed a procedure for manufacturing high-flavored butter. The procedure consists of the following steps. Two cultures are used in this process: an L culture (culture containing *Leuconostoc*) designated Fr 19, and the D culture (culture containing *diacetylactis* strain, currently called Cit$^+$ *Lactococcus*

lactis ssp. *lactis*) NIZO 4/25. Both cultures are grown separately in 16% (w/v) reconstituted skim milk for 18 to 22 hours at 21°C (69.8°F). The material cultured with 4/25 is then mixed with lactic permeate at a ratio of 2:3 to reduce the pH from about 4.9 to 3.2. The lactic permeate is made by fermenting whey with a strain of *Lactobacillus helveticus,* centrifuging the cells and any sediment out, and concentrating the supernatant to obtain a lactic acid level of about 12%. After acidification, the 4/25 cultured material is vigorously aerated for 30 minutes, during which the α-acetolactate generated is converted into high levels (about 150 mg/ml or 15%) of diacetyl. This material containing the high concentration of diacetyl is mixed with a sufficient amount of milk cultured with Fr 19 and is worked into butter to give a diacetyl concentration of about 2 micrograms (2 ppm)/g of butter. The culture Fr 19 is needed to remove the green taint introduced by the acetaldehyde generated by the *diacetylactis* component in NIZO 4/25. This process is used by a large commercial company that produces butter and margarine in Europe and the United States.

A modification of the NIZO 4/25 procedure was adapted for large-scale fermentation involving continuous feeding of citrate (as an additional diacetyl precursor), mechanical aeration and agitation and stabilization of high concentration α-acetolactate, and subsequent drying to a powder with high flavor-generating potency. The high levels of diacetyl precursors in the powder, when used in various dairy products, confectionaries, and dressings, are converted into diacetyl during heat-processing and agitation used in the manufacture of these products. Such powders are currently available (Kerry 2004c).

Powders made from large-scale yogurt fermentations in which the desired acetaldehyde concentrations are stabilized for commercial spray-drying are also available for dry dairy and baking mixes in which yogurt flavor is desired (Kerry, 2004d). The detailed manufacturing processes for these products are proprietary.

Intense Dairy Flavors and Flavor Blocks

Composite intense dairy flavors are produced by the combined use of selected microorganisms used in food fermentations and enzymes. Seitz (1990), in his excellent review, refers to these products "as flavor bases or complex flavoring materials containing volatiles, nonvolatiles or both." Such flavorings are used in compounded flavors to impart desirable flavoring in cheese foods, processed cheese, baked products, convenience foods, butter substitutes, confectionaries, etc. Dairy substrates used for such flavor bases include casein, butterfat, and cheese (and cheese slurries). Sometimes flavor bases derived from non-dairy substrates are combined with those derived from dairy substrates to formulate cost-effective, highly intense compounded flavors that may be used at low levels (about 0.1% to 0.5%) in foods that require dairy flavors.

The production of such intense flavors is a multi-step process, as outlined by Seitz (1990), that requires sensory evaluation of the flavoring at various stages of development at appropriate dilutions in food systems such as cheese sauce, milk, acid-sugar solutions, etc. Such evaluations yield important information on the flavor characteristics, intensities, and cost/use that is needed for commercial applications. For a fairly detailed discussion of dairy flavor bases, see Seitz (1990).

In many instances, such complex flavoring bases cannot be produced as a single, composite entity. In such cases separate flavor blocks are skillfully combined to create a compounded flavor base. The basic flavor blocks known in the flavoring trade include umami (or savory), kokumi sensation, sharpness or bite (also called piquant), and bitterness. Other blocks needed in dairy

applications include "sweetish, creamy/buttery" notes and "sulphury" notes. While these bases make up the major portion of the flavor desired, the intense and specific flavor attributes may only be achieved by formulating a WONF (with other natural flavors). This involves the careful selection and proportioning of fermentation-derived products to give the top notes needed in a finished flavor.

The addition of key intermediates such as succinic acid is necessary in the development of intense dairy flavors. Several important flavor compounds are derived from this key tricarboxylic acid cycle molecule. Certain *Lactobacillus reuteri* strains have been found to convert citric acid or its salts efficiently to succinic acid. Such biological conversions aid in the development of intense dairy flavor blocks (Seitz, 1991). Other important flavor compounds needed in dairy flavors consist of different esters, which impart the fruity notes needed in dairy and food flavors. Such molecules may be derived by fermentating branched chain amino acids by dairy-derived yeasts. Farbood et al. (1986) patented a successful process for such conversions for industrial scale applications.

The various microorganisms and enzyme systems involved in the development of such flavorings are discussed in reviews by Seitz (1990) and Vedamuthu (1979, 1988).

Purified Bio-flavorings

Starter distillates, derived from culturing dairy substrates with selected flavor bacterial combinations, are used to flavor or boost flavor in butter, margarine, and dairy desserts, puddings, and confectionaries. Starter distillates contain the volatile flavor compounds generated in suitable dairy substrates using selected high flavor-generating lactic cultures. Optimum pH and temperature are maintained in these fermentations with continuous replenishment of citrate. At appropriate stages the volatiles are stripped off by distillation at minimum temperatures necessary for volatilizing the essential flavor compounds and trapped in a suitable solvent. Starter distillates contain not only diacetyl but also other balancing and complementing flavor compounds to impart a full flavor.

Seitz (1990) has provided an extensive review of starter distillates. The concept of starter distillates was introduced by Ruehe (1938). He proposed the idea of using starter distillates to eliminate variations in the intensity of flavor commonly encountered from batch to batch in creamery butter. The variability could be traced to inherent differences in the performance of starter cultures, the differences in citrate concentrations in cream seasonally and through the course of the lactation period, and day to day variations in production conditions in the creamery. Furthermore, with the introduction of sweet cream for butter making, a reliable method for ensuring uniform, high flavored butter was needed to avoid keeping quality issues linked to ripened cream. Starter distillates that were standardized to deliver the needed flavor fulfilled the demand.

In 1938, Ruehe published his procedure for producing starter distillate. It called for careful selection of a mixed-strain dairy starter containing mesophilic flavor bacteria, and meticulous culture maintenance. The starter was propagated in a milk-based medium fortified with 0.15$ to 0.3% citric acid. At the appropriate stage ferric chloride was added to the culture to oxidize the acetoin in the culture to diacetyl, and the volatiles were steam distilled. The distillate was then standardized to a diacetyl concentration of 1,000 mg/L. Ruehe (1938) pre-empted the filing of patents on starter distillates by placing this technology in the public realm. Seitz (1990) has summarized several variations of the process that have been published on starter distillates.

Starter distillates in liquid and powdered forms have long been marketed by the leading

culture house, Chr. Hansen Laboratories. Other companies, including International Flavors and Fragrances (IFF), also offer a variety of such flavor distillates and essences for dairy and food applications.

Bio-preservatives

Over the past two decades, there has been intense research interest in bacteriocins produced by lactic acid and related bacteria for application as preservatives in foods. Another area that has attracted equal interest has been the use of acidic metabolic byproducts of lactic acid fermentation for preservative application in foods. The preservative effect of lactic acid against Gram-negative spoilage bacteria has been known for a long time. Therefore, the inhibitory effect of acetic acid against *Pseudomonas* and *Bacillus* species and the suppressive activity of propionate against molds and certain yeasts is common knowledge.

Whey-based fermentation has been used to produce an acetate-propionate mixture, which is then dried to yield powders containing high concentrations of the acids. In this process, lactose in whey is converted to lactic acid by fermenting with high-temperature *Lactobacillus* species or a combination of *Streptococcus thermophilus* and a suitable *Lactobacillus* species (for example, *Lactobacillus delbrueckii* or *Lactobacillus acidophilus*). The lactic acid liquor is subsequently fermented with selected strains of *Propionibacterium* sp. At the end of the fermentation, the entire fermentation liquid is spray dried to obtain powders containing acetate and propionate concentrations as high as 16% acetate and 30% to 32% propionate. These powders may be used to control Gram-negative spoilage bacteria as well as molds and certain yeasts in dairy products such as cottage cheese, yogurt, sour cream, etc. The powders also may be used in combination with anti-caking agents to control molds in shredded cheese. This product is commercially available and widely used in the industry (Kerry, 2004e).

MicroGARD™ is another product that is produced by fermentation of whey, milk, or whey-milk mixtures with *Propionibacterum* species. It has been on the market for some time. This product originally was sold in liquid form, but now is available in powdered form. The product has been widely used in the industry to control Gram-negative spoilage flora, yeasts, and molds in yogurt, cottage cheese, and other foods. It originated from research conducted at Oregon State University, which culminated in the issue of a patent assigned to the state of Oregon (Ayers et al. 1992).

The major thrust of this discovery relates to the unexpected activity of *Propionibacterium* fermentation liquids against Gram-negative spoilage bacteria and food spoilage yeasts. Although propionic acid is known to be antagonistic to molds at relatively low concentrations, levels as high as 2% are needed to inhibit yeasts. Such a high level of propionate introduces undesirable flavors and taints in foods. In most *Propoinibacterium* fermentations the concentrations of propionic acid and acetic acid range between 0.8% and 1.3% and 0.35% and 0.6%, respectively. The patent describes various examples in different dairy and food products in which the inhibition exerted by *Propionibactrium* fermentation liquids before and after drying could not be attributed to propionate-acetate alone. Further analyses of *Propionibacterium* fermentation liquids showed that a proteinaceous product with a molecular weight greater than 300, in combination with the organic acid components, is associated with such unexpected inhibitory activity against spoilage-inducing Gram-negative bacteria and yeasts.

MicroGARD™ is sold by Danisco Corporation. They market several products under this label, including MG 100, MG 200, MG 300, MG 400, and MG CS 1-50. For further details on these products, consult the relevant trade literature (Danisco, 2005a).

Microbial Metabolites Containing Bacteriocins

Bacteriocins are ribosomally synthesized proteinceous substances or peptides produced by bacteria that inhibit other bacterial species or strains. Lactic acid bacteria commonly used in starters and found in fermented dairy products produce bacteriocins. Many of the species and strains that belong to the genera *Lactococcus, Lactobacillus, Streptococcus, and Pediococcus* elaborate bacteriocins. These bacteriocins normally have a narrow spectrum of activity against closely related strains or species. However, certain bacteriocins of lactic acid bacteria have a broader spectrum of activity against bacteria that cross the species and even the generic lines. A good example of such a bacteriocin is nisin, which is produced by certain strains of *Lactococcus lactis* ssp. *lactis.* Nisin is active against closely related lactococci, many lactobacilli, streptococci, and pediococci, as well as *Listeria* sp., and several spore-forming *Bacillus* sp. and *Clostridium* sp.

Pediococci, often encountered in cheeses, produce bacteriocins called pediocins. Pediocins also have a wide spectrum of activity, affecting the growth of other closely related pediococci, many lactobacilli, and *Listeria* sp. However, they have no inhibitory activity against dairy lactococci.

All of the aforementioned bacteria belong to the Gram-positive group. Generally, bacteriocins derived from Gram-positive bacteria do not affect Gram-negative bacteria.

The widespread food- and dairy-related outbreaks of *Listeria monocytogenes* in several parts of the world over the past two decades has led to a clamor for finding suitable control measures for containing listerial food-borne outbreaks. A breakthrough for controlling *Listeria monocytogenes* came about when Gonzalez and Kunka (1987) reported on the production of a bacteriocin active against several Gram-positive species by a strain of *Pediococcus acidilactici* designated PAC 1.0. The production of the bacteriocin (designated PA-1) was coded on a 9.4-kbp plasmid designated pSRQ 11. Later, Pucci et al. (1988) demonstrated that pediocin PA-1 inhibited *Listeria monocytogenes* in broth, agar plate, and food systems, including cottage cheese.

Based on the findings of Pucci et al. (1988), a patent was issued to Vandenbergh et al. (1990), wherein they described the use of dried metabolites from *Pediococcus acidilactici* PAC 1.0 to control *Listeria* in food systems. Henderson et al. (1992) extended the study on pediocin PA-1 by describing its purification and primary structure. Later, Marugg et al. (1992) elucidated the nucleotide sequence of the genes involved in the production of pediocin PA-1. As knowledge of the bacteriocin accumulated, technology for industrial-scale production of the bacteriocin and for drying the "fermentate containing pediocin PA-1" was developed.

Currently, standardized dried powders containing the bacteriocin (pediocin PA-1) and other active acidic metabolites are commercially available for controlling *Listeria* in dairy foods. The acidic metabolite components are considered to act synergistically with the bacteriocin in controlling the intended targets (Kerry, 2004f). A similar product containing metabolites such as organic acids and peptides derived from the fermentation of a dairy *Lactococcus* strain exhibiting inhibitory activity against several Gram-positive bacteria is also currently available on the market for use in dairy products (Kerry, 2004g).

Purified Bacteriocin

Among the various bacteriocins elaborated by lactic acid bacteria, only nisin in purified form has been used in food products. Hirsch (1951) first suggested the use of nisin in food products.

The term nisin denotes a family of inhibitory polypeptide molecules elaborated by

Lactococcus lactis subsp. *lactis* strains. The active protein with a mass of 3,500 Da exists in multimeric form. This bacteriocin is ribosomally synthesized as a prepeptide (57 amino acid peptide) and posttranslationally processed into a mature peptide (34 amino acid peptide). The genetic determinants for nisin production are encoded on a transposon called Tn5301. Nisin contains three unusual amino acids: dehydroalanine, lanthionine, and β-methyl lanthionine; thus, nisin is called a lantibiotic.

Genes associated with nisin synthesis are nisA, nisB, nisT, and nisC. nisA is the structural gene for the prepeptide,; nisB codes for a membrane-associated protein, nisT is considered to be involved in the transport of nisin, and nisB has been found to bear homology to proteins involved in the biosynthesis of a closely related bacteriocin called subtilin (also a lantibiotic) produced by certain strains of *Bacillus subtilis* (Vandenbergh, 1993).

Nisin interacts with cytoplasmic membranes of susceptible bacteria and forms potential dependent pores that cause the destruction of proton-motive force, thus permitting the efflux of ions, small molecules, and cytoplasmic components. Although the binding of nisin to the cell walls of sensitive cells is known, the exact mechanism by which it destabilizes the cytoplasmic membranes is not yet understood (Vandenbergh, 1993).

As mentioned earlier, nisin has a broad inhibitory spectrum. It is active against species belonging to the following genera: *Lactococcus, Streptococcus, Micrococcus*, *Staphylococcus, Pediococcus, Lactobacillus, Listeria, Mycobacterium, and Bacillus,* including their spores, and *Clostridium* and their spores. It is inactive against Gram-negative bacteria, but in the presence of chelating agents such as EDTA, nisin has been shown to be active against *Salmonella, Escherichia coli, Pseudomonas aeruginosa,* and certain other Gram-negative species.

In 1959 nisin was permitted as a preservative in the United Kingdom. Initially, it was used to prevent clostridia-induced blowing of high moisture cheese. Also in 1959, nisin was accepted as a food additive by the Joint Food and Agriculture Organization and World Health Organization of United Nations. It is currently accepted as a legal food additive worldwide. Application studies in the use of nisin as a preservative in various dairy foods have been reported in the literature. Vandenberg (1993) has provided a concise summary of the use of nisin in dairy foods and its regulatory status in various countries.

Nisin is currently marketed under the trade name Nisaplin® by Danisco for application in dairy foods. For more information, see the trade bulletin by Danisco (2005c).

Bioactive Peptides and Nutraceuticals

The term functional foods has gained widespread currency in food scientific literature. Functional foods contain health-promoting components beyond traditional nutrients, referred to as nutraceuticals. Among the nutraceuticals, bioactive peptides have garnered great interest in therapeutic and preventive nutrition.

Casein and whey proteins are rich in bioactive peptides. These peptides are physiologically inactive in the native milk proteins, but bioactive peptides are released when the proteins are hydrolyzed. These peptides display biological activity ranging from antihypertensive effects to immunostimulation and opioid agonistic effects. Casokinins, β-lactorphins, α-lactorphins, and lactkinins are bioactive peptides from casein and whey proteins that exhibit inhibitory activity against angiotension-I and -II-converting enzymes, which are involved in causing hypertension. The angiotension-I-converting enzyme is referred to as ACE, and the bioactive peptides counteracting the activity of ACE are called ACE inhibitors.

ACE-inhibitors are released during dairy fermentation by lactic acid bacteria from milk proteins. Several lactobacilli and some lactococci used in dairy fermentations are known to release these bioactive peptides (Gobbetti et al., 2007). Thus, intake of fermented dairy products serves as intake of natural functional foods for controlling hypertension. A probiotic culture that is said to possess ACE-inhibitory activity is being marketed by a leading starter culture company.

Conjugated linoleic acids (CLA) is another class of dairy-derived components with health implications. These fatty acids are synthesized by the rumen flora, which are incorporated into the lacteal fluid during the synthesis of milk. Milk fat contains the major portion of CLA, ranging from 2.4 to 28.1 mg/g of fat. Yogurt is a good source of CLA. The most biologically active CLA isomer found in dairy products is *cis*-9, *trans*-11–18:2. CLAs are strong antioxidants and may prevent colon and breast cancer. They have been shown to enhance immune response and reduce the levels of prostaglandin PGE-2, which is involved in heart disease. Chandan and Kilara (2007) have provided an in-depth discussion of these dairy components, which in the coming years will occupy prominent niches in the nutraceutical marketplace.

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Chapter 15

Dairy-based Ingredients: Regulatory Aspects

Dilip A. Patel

Introduction

The last two decades have seen considerable growth in dairy ingredient manufacture as evidenced in trade shows and professional meetings. Over and above product formulation and standardization needs, there is an emerging trend toward product functionality. In a technical sense, functionality is aimed at improving body, texture, structure, emulsion, or suspension in a product. However, in recent years, various ingredient applications are increasingly sought adding value to food products beyond normal nutrition or technical function, i.e., nutraceutical, bioactive, or functional food attributes.

In recent years, several recalls associated with faulty labeling have spurred attention on food ingredients. Understanding regulatory aspects has become increasingly important to achieve allergen management as well as comply with legal obligations encompassing dairy ingredients.

This chapter highlights regulatory related aspects of dairy-based ingredients in the United States. The following aspects are discussed: product identity, nutritional labeling, and allergen declaration; current good manufacturing practices and dairy HACCP; food additives and "generally recognized as safe"; and the new ingredient approval process.

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Product Identity, Nutritional Labeling, and Allergen Declaration

Regulations concerning product identity (product-specific Codes of Federal Regulation specifying intended use) cover approved ingredients as well as ingredients declaration, including allergen-related information (Nutrition Labeling and Education Act). Key provisions regulating dairy ingredients are summarized in Tables 15.1 and 15.2.

Current Good Manufacturing Practices, Dairy Hazard Analysis and Critical Control Points, and Pasteurized Milk Ordinance

The current good manufacturing practices (GMP) ideology originated from U.S. food and drug legislation and the regulatory framework. Presence of harmful substance(s) in food is considered adulteration and is punishable by civil and/or criminal penalties, depending upon intent, liability, negligence, and reasonable effort aspects adjudicated by case law. GMP guidelines provide a basic framework of the requirements for production of hygienic foods for human consumption. GMP guidelines, in one form or another, serve as a model food code for food operations in the United States as well as around the world (Patel et al., 2008). GMP have continued to evolve to reflect current needs and proactive responsible governance. The guidelines are currently undergoing revision

Table 15.1. Resources for key provisions regulating dairy ingredients in the United States.

Provision	Basis/agency recommendation
Standards of identity	See Table 15.2
Food labeling	21 CFR: 1B: Part 101
Nutritional quality guidelines for foods	21 CFR: 1B: Part 104
Current good manufacturing practices in manufacturing, packing, or handling food for human consumption	21 CFR: 1B: Part 110
Pasteurized Milk Ordinance	U.S. Department of Health and Human Services, public health services, and U.S. Food and Drug Administration

Source: Patel, Oliver, Almeida and Vedamuthu, 2008.Compiled from various sources.

Table 15.2. Resources for dairy ingredients standards of identity in the United States.

Product/process	Legal Basis
Pasteurization	21 CFR 58.334
Whey	21 CFR 58.2601
Dry buttermilk and dry buttermilk product	21 CFR 58.2651
Definitions: cream, pasteurized, and ultra-pasteurized	21 CFR 131.3
Milk	21 CFR 131.110
Acidified milk	21 CFR 131.111
Cultured milk	21 CFR 131.112
Concentrated milk	21 CFR 131.115
Sweetened condensed milk	21 CFR 131.120
Low-fat dry milk	21 CFR 131.123
Nonfat dry milk	21 CFR 131.125
Nonfat dry milk fortified with vitamins A and D	21 CFR 131.127
Dry whole milk	21 CFR 131.147
Dry cream	21 CFR 131.149
Heavy cream	21 CFR 131.150
Light cream	21 CFR 131.155
Light whipping cream	21 CFR 131.157
Sour cream	21 CFR 131.160
Acidified sour cream	21 CFR 131.162
Boiler water additives	21 CFR 173.310
Whey	21 CFR 184.1979
Concentrated whey	21 CFR 184.1979(2)
Dried or dry whey	21 CFR 184.1979(3)
Reduced lactose whey	21 CFR 184.1979a
Reduced minerals whey	21 CFR 184.1979b
Whey protein concentrate	21 CFR 184.1979c

Patel, Oliver, Almeida, and Vedamuthu (2008). Based on the Federal Food, Drug and Cosmetic Act, as amended. Sec. 402 [342]
Adulterated Food and Sec. 403 [343] Misbranded Food.

Table 15.3. Resources on fundamental elements of GMP in the United States.

21 CFR Part 110	Focus area
Section 110.3	Definitions
Section 110.5	Current good manufacturing practice
Section 110.10	Personnel
Section 110.19	Exclusions
Section 110.20	Plants and grounds
Section 110.35	Sanitary operations
Section 110.20	Sanitary facilities and controls
Section 110.40	Equipment and utensils
Section 110.80	Processes and controls
Section 110.93	Warehousing and distribution

Patel, Oliver, Almeida, and Vedamuthu (2008)

to reflect the needs of the 21st century. Principles and essential elements of GMP are depicted in Table 15.3.

A comprehensive revision of GMP is ongoing as a continuous exercise to modernize and enhance the effectiveness and relevance of current laws. An approach based on risk analysis is the defining characteristic in the revised version. According to the U.S. Food and Drug Administration (FDA), modernized current GMP facilitate control of food safety hazards for food manufacturers by logical allocation of resources with maximum impact on food safety. Improved measures suggested in the amended regulation are based on risk analysis and they have been proven to significantly reduce the risk of foodborne hazards.

Salient aspects of current GMP are as follows:

- Risk-based approach requiring integration of regulatory requirements with food safety outcomes
- Appropriate training
  - * Specific training emphasizing hygiene and sanitation (personal as well as prem-

ises, surroundings, and operational territories) in food protection
- Food allergen control plan
  * The manufacturer must address six elements
    — Training of processing and supervisory personnel
    — Segregation of food allergens during storage and handling
    — Validated cleaning procedures for food contact equipment
    — Prevention of cross-contact during processing
    — Product label review and label usage and control
    — Supplier control program for ingredients and labels
- Written environmental pathogen control program for production of ready-to-eat (RTE) foods that support growth of *Listeria monocytogenes* (LM)
- Written sanitation procedures
  * Food processors are required to develop and maintain written sanitation procedures that define the scope, sanitation objective, management responsibility, monitoring, corrective action, and record keeping associated with the sanitation procedure
- Maintenance of critical records

Dairy Hazard Analysis and Critical Control Points and Pasteurized Milk Ordinance

Systems based on dairy hazard analysis and critical control points (HACCP) and the Pasteurized Milk Ordinance (PMO) address industry requirements related to dairy food safety. The National Conference on Interstate Milk Shipments (NCIMS) promotes dairy HACCP (grade A voluntary HACCP) to enhance food safety in dairy processing operations.

According to Howard Bauman (1974, 1995), HACCP is a preventive system of control based on a rational and logical process of estimating the risk associated with producing and marketing a given food product. Control of food processing may be obtained and maintained through diligent, intelligent application of the principles of hazard analysis and the identification of control points that are critical to food safety (Bauman, 1974, 1995). According to an advisory committee on microbiological criteria, HACCP is a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production to procurement, handling, manufacturing, distribution, and consumption of the finished product. HACCP is a common-sense, practical, and achievable food safety approach that industry strives to follow, within the limitations of the available technology to produce, transport, procure, and prepare foods that present a minimum level of risk from foodborne hazards (Bauman, 1974).

While dairy HACCP is relatively recent to the dairy processing industry, the PMO has been in place since 1926. The fundamental basis of the PMO has been to encourage uniformity and a higher level of milk sanitation excellence in the United States. It encourages legal adaptation by states, counties, and municipalities to facilitate shipment and acceptance of milk and milk products of high sanitary quality in interstate and intrastate commerce (FDA, 2003). This ordinance has been widely adapted and has been upheld by court decisions since its implementation. Key provisions underlying the PMO and related standards are given in Table 15.4. For more detailed information, see the PMO document at www.cfsan.fda.gov/~ear/pmo03toc.html.

The unique feature of the PMO document is its public health reason explanatory notes, in which scientific rationale and practical knowledge accumulated over many years are presented accurately and clearly. The document is user-friendly and serves as an excellent training resource.

Table 15.4. PMO standards for milk and milk products.

Grade "A" raw milk and milk products for pasteurization, ultrapasteurization, or aseptic processing	Temperature	Cooled to 10°C (50°F) or less within 4 hours or less of the commencement of the first milking, and cooled to 7°C (45°F) or less within 2 hours after the completion of milking, provided that the blend temperature after the first milking and subsequent milkings does not exceed 10°C (50°F)
	Bacterial limits	Individual producer milk not to exceed 100,000/mL prior to commingling with other producer milk; not to exceed 300,000/mL as commingled milk prior to pasteurization
	Drugs	No positive results on drug residue detection methods as referenced in Section 6-Laboratory
	Somatic cell count*	Individual producer milk not to exceed 750,000/mL
Grade A pasteurized milk and milk products and bulk shipped, heat-treated milk products	Temperature	Cooled to 7°C (45°F) or less and maintained at that temperature
	Bacterial limits**	20,000/mL or gm***
	Coliform****	Not to exceed 10/mL, provided that in the case of bulk milk transport tank shipments, it shall not exceed 100/mL
	Phosphatase*****	Less than 350 milliunits/L for fluid products and other milk products by the fluorometer or Charm ALP or equivalent
	Drugs**	No positive results on drug residue detection methods as referenced in Section 6-Laboratory techniques which have been found to be acceptable for use with pasteurized and heat-treated milk and milk products
Grade A pasteurized concentrated (condensed) milk and milk products	Temperature	Cooled to 7°C (45°F) or less and maintained there unless drying is commenced immediately after condensing
	Coliform	Not to exceed 10/gram, provided that in the case of bulk milk transport, tank shipments shall not exceed 100/ml.
Grade A aseptically processed milk and milk products	Temperature	None
	Bacterial limits	Refer to 21 CFR 113. 3(e)(1)******
	Drugs**	No positive results on drug residue detection methods as referenced in Section 6-Laboratory techniques that have been found to be acceptable for use with aseptically processed milk and milk products
Grade A nonfat dry milk		No more than:
	Butterfat	1.25%
	Moisture	4%
	Titratable acidity	0.15%
	Solubility index	1.25 m.
	Bacterial estimate	30,000/gram
	Coliform	10/gram
	Scorched particles disc B	15/gram
Grade A whey for condensing	Temperature	Maintained at a temperature of 45°F (7°C) or less, or 63°C (145°F) or greater, except for acid-type whey with a titratable acidity of 0.40% or above or a pH of 4.6 or below
Grade A pasteurized condensed whey and whey products	Temperature	Cooled to 7°C (45°F) or less during crystallization within 48 hours of condensing
	Coliform limit	Not to exceed 10/gram
Grade A dry whey, grade A dry whey products, grade A dry buttermilk, and grade A dry buttermilk products	Coliform limit	Not to exceed 10/gram

Patel, Oliver, Almeida, and Vedamuthu (2008)
*Goat milk 1 million/mL
**Not applicable to acidified or cultured products
***Results of the analysis of dairy products that are weighed to be analyzed are reported in #/gm
****Not applicable to bulk shipped heat-treated milk products
*****Not applicable to bulk shipped heat-treated milk products; ultrapasteurized products that have been thermally processed at or above 138°C (280°F) for at least 2 seconds to produce a product that has an extended shelf life (ESL) under refrigerated conditions; and condensed products
******21 CFR 113.3(e) (1) contains the definition for "commercial sterility"

Implementing HACCP

HACCP implementation is a systematic exercise of identifying, evaluating, and controlling food safety hazards based on the following essential principles (FDA, 1997).

Principle 1: Conduct a hazard analysis.

Principle 2: Determine the critical control points (CCPs).

Principle 3: Establish critical limits.

Principle 4: Establish monitoring procedures.

Principle 5: Establish corrective actions.

Principle 6: Establish verification procedures.

Principle 7: Establish record-keeping and documentation procedures.

Prerequisite programs (PPs) should be in place to ensure successful HACCP implementation. CGMP, PMO, and the Food Code are good guides for implementing PP. According to the FDA, the important characteristics of effective PPs are:

- Sanitary establishments and facilities with linear product flow and logical traffic patterns that minimize cross contamination from raw to processed products
- A well-executed supplier assurance and verification program
- Clear and regularly updated ingredient, product, and packaging material specifications that reflect known concerns
- Sanitary processing equipment with preventive maintenance and regular calibration schedules
- Written cleaning and sanitation procedures with an aggressive verification schedule
- Adequate employee hygiene and training; look for problem areas and continuously innovate to address this important issue
- Pest control and non-food chemical hazard control
- Well-rehearsed recall and traceability procedures
- Logical standard operating procedures (SOPs) for processes, product formulations, sanitation, and allergen control
- Proactive glass and metal control programs
- Organized shipping, receiving, and storage procedures and logistics

Although prerequisite programs may impact HACCP, they are designed to ensure wholesomeness and suitability of food for human consumption. In contrast, HACCP plans are narrower in scope and specific to ensuring food safety. Prerequisite plans should be in place and their effectiveness assessed as HACCP plans are being designed and implemented. All PP should be documented and regularly audited.

Food Additives and "Generally Recognized as Safe"

Within the FDA, the Center for Food Safety and Applied Nutrition's (CFSAN) Office of Food Additive Safety is responsible for reviewing safety information for food ingredients and food packaging. The Food Additives Amendment to the Federal Food, Drug, and Cosmetic (FD&C)Act was enacted in 1958. This act defined the term food additive and emphasized premarket approval for new uses of food additives. The law established the standard of review, the standard of safety, and formal rule making procedures for food additives. Substances excluded from the definition of food additives are those that are generally recognized among experts qualified by scientific training and experience to evaluate their safety as having been adequately shown to be safe under the conditions of their intended use. In other words, substances that are generally recognized as safe (GRAS) under conditions of their intended use are not food additives and do not require premarket approval by FDA (Gaynor, 2005-06).

The following are important FDA terminologies concerning food additives.

GRAS is an acronym for "generally recognized as safe." Under sections 201(s) and 409 of the FD&C Act, any substance that is intentionally added to food is a food additive which is subject to premarket review and approval by the FDA, unless the substance is generally recognized among qualified experts as having been adequately shown to be safe under the conditions of its intended use, or unless the use of the substance is otherwise excluded from the definition of a food additive.

Food additive is defined by the FDA as any substance whose intended use results or may reasonably be expected to result, directly or indirectly, in its becoming a component of or otherwise affecting the characteristic of any food if such substance is not GRAS or sanctioned prior to 1958 or otherwise excluded from the definition of food additives. The components include any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food, including any source of radiation intended for any such use. In 1958, FDA published a list of GRAS substances and incorporated the list in Title 21 of the Code of Federal Regulations. The current list appears in 21 CFR Parts 182, 184, and 186.

Food contact substance is defined as a substance that is intended for use as a component of materials used in manufacturing, packing, packaging, transporting, or holding food if such use of the substance is not intended to have any technical effect in such food. A food contact substance (FCS) is a single substance such as a polymer or an antioxidant in a polymer. As a substance, it is reasonably pure (as per the chemists' definition of substance). Although a polymer may be comprised of several monomers, it still has a well-defined composition.

Food contact material (FCM) is made with an FCS, usually with other substances. It is often (but not necessarily) a mixture such as an antioxidant in a polymer. The composition of FCM may be variable.

Food contact article is the finished film, bottle, dough hook, tray, etc. that is formed out of the FCM.

Indirect food additives are food additives that come into contact with food as part of packaging, holding, or processing, but are not intended to be added directly to, become a component of, or have a technical effect in or on the food. Indirect food additives mentioned in Title 21 of the U.S. Code of Federal Regulations (21 CFR) used in food-contact articles include adhesives and components of coatings (Part 175), paper and paperboard components (Part 176), polymers (Part 177), and adjuvants and production aids (Part 178). Currently, additional indirect food additives are authorized through the food contact notification program. In addition, indirect food additives may be authorized through 21 CFR 170.39.

Prior sanctioned substance is a substance whose use in or on food is the subject of a letter issued by FDA or the U.S. Department of Agriculture (USDA) offering no objection to a specific use. The prior sanction exists only for a specific use of a substance in food delineating level(s), condition(s), and product(s) set forth by explicit approval by FDA or USDA prior to September 6, 1958.

Threshold of regulation (TOR) exemption is a specific exemption for a substance used in a food contact article that may be exempted from the requirement of a food additive listing regulation if the use in question has been shown to meet the requirements in 21 CFR 170.39.

In the case of food additives, the FDA determines the safety of the ingredient; however, a determination that an ingredient is GRAS also can be made by qualified experts outside of government. This is permitted by the so-called GRAS affirmation (until 1997), now known as GRAS notifica-

tion (62 FR 18938; April 17, 1997). Under 21 CFR 170.30(b), general recognition of safety through scientific procedures requires the same quantity and quality of scientific evidence as is required to obtain approval of the substance as a food additive and ordinarily is based upon published studies, which may be corroborated by unpublished studies and other data and information.

Typically, industry submits the GRAS notice under the GRAS notification procedure. The FDA evaluates whether each submitted notice provides a sufficient basis for a GRAS determination and whether information in the notice or otherwise available to FDA raises issues that lead the agency to question whether use of the substance is GRAS. The FDA responds to the notifier by letter (FDA, 2009). Among the ingredients approved by the GRAS mechanism are canola oil, enzyme preparations, whey, cocoa butter substitute, and phytosterols.

While preparing the GRAS notification, it is important to remember the following points. Regardless of whether the use of a substance is a food additive or GRAS, there must be evidence that the substance is safe under the conditions of its intended use. FDA has defined safe (21 CFR 170.3(i)) as a reasonable certainty in the minds of competent scientists that the substance is not harmful under its intended conditions of use. The specific data and information that demonstrate safety depend on the characteristics of the substance, the estimated dietary intake, and the population that will consume the substance.

For a food additive, privately held data and information about the use of the substance are sent by the sponsor to the FDA, which evaluates those data and information to determine whether the substance is safe under the conditions of its intended use (21 CFR 171.1). For a GRAS substance, generally available data and information about the use of the substance are widely known and accepted by qualified experts, and there is a basis to conclude that there is consensus among qualified experts that those data and information establish that the substance is safe under the conditions of its intended use.

New Ingredient Approval Process

Any food additive intended to have a technical effect in food and any color additive for use in foods, drugs, cosmetics, or medical devices that are in contact with the body for a significant period of time is deemed unsafe unless it conforms to the terms of a regulation prescribing its use or to an exemption for investigational use. A petition for a food additive or color additive is submitted to request issuance of a regulation allowing new uses of the additive, and must contain the necessary supporting data and information.

Prior to submitting a petition, it may be useful to verify that the candidate ingredient is not already regulated for the intended use by consulting the FDA's regulations in Title 21 of the CFR, parts 170 to 199 for food additives, and parts 73 and 74 for color additives. In addition, the FDA has a nice resource titled *Everything Added to Food in the United States* (EAFUS). If a food additive is regulated for use in food, it is listed in EAFUS with a "regnum" (a regulation in 21 CFR). To be acceptable for a particular application, an additive must meet the identity, specifications, and use limitation provisions in the applicable regulation.

The following administrative steps are involved in a new food additive approval cycle (Figure 15.1):

1. Data are submitted by the petitioner
2. There is communication between the FDA and the petitioner
3. The FDA reviews the data as clarified by the communication and prepares memoranda
4. The FDA publishes a final regulation, which authorizes a specific use of the additive, must withstand legal challenge, and bears FDA's credibility.

A new petition is received.
↓

- OFAS evaluates the petition for adequacy of filing (if adequate, a filing notice is published in the Federal Register).
- The need for review outside CFSAN is determined.

↓

The consumer safety officer distributes appropriate parts of the petition to experts for evaluation:
↓

Chemistry review	Toxicology review	Environ-mental review	Evaluation by other government experts	Evaluation by outside (nongovernment) experts
The identity, specifications, manufacturing, use, functionality, and exposure estimate are verified. Analytical methods are evaluated.	Safety studies are evaluated.	Environmental data are evaluated.	As needed on a case-by-case basis.	As needed on a case-by-case basis.
Probable exposure is calculated.	Acceptable exposure level is determined.	An environmental memo is prepared.	A report is prepared.	A report is prepared.

↓

Decisions are made:
- A tentative safety conclusion is reached.
- Advisory committee input is received, if necessary.
- The final safety evaluation is made.
- The administrative record is compiled.
- A Federal Register final rule is drafted.

↓

The final agency review is completed.

The final regulation is published in the Federal Register.

The regulation appears in the Code of Federal Regulations.

Figure 15.1. Administrative steps in a new food additive approval cycle.

According to the FDA, the basic elements of a safety assessment for an additive are as follows:

1. Identity
2. Probable exposure
3. Evaluation of safety
4. Limitations of conditions of use (may be necessary to ensure safe use)

According to the FDA, the following are essential elements of a food and/or color additive petition:

- The identity and composition of the additive
- Proposed use
- Use level
- Data establishing the intended effect
- Quantitative detection methods
- Estimated exposure from the proposed use (in food, drugs, cosmetics, or devices, as appropriate)
- Full reports of all safety studies
- Proposed tolerances (if needed)
- Environmental information (as required by the National Environmental Policy Act; NEPA), as revised (62 FR 40570; July 29, 1997)

Ensure that consistent information is presented throughout all sections of the petition, including those pertaining to chemistry, toxicology, environmental science, and any other pertinent studies (e.g., microbiology).

For a food additive petition, the following is recommended by FDA to be appropriate chemistry information:

- The design of any study and assessment of the results should be based on sound scientific principles, not rote adherence to guidelines.
- Significant deviations from appropriate guidelines should be justified and possible effects on the study discussed.
- Consult with the Office of Food Additive Safety (OFAS) for protocol review before initiating any studies to ensure:
  * That the proper test data will be submitted
  * That the analytical methodology will be adequate
  * That the validation methodology will be adequate
- Submit all supporting raw data, including that for:
  * Samples
  * Standards
  * Construction of calibration curves
  * Determination of limits of detection (LOD) and limits of quantitation (LOQ)
- Submit a complete description of the analytical methods, including:
  * Sample workup
  * Preparation of standards
  * Example calculations
- Submit all other relevant information, for example:
  * Technical brochures
  * Material safety data sheets (MSDS)
  * Methodology and calculations for estimating intake
  * References, as appropriate (in English)
- Discuss the results: interpretations and conclusions should be scientifically sound and supported by the data.
- Do not make any unsupported statements.

According to an FDA guidance document, information allowing the unequivocal identification and characterization of the food additive should be provided. Such items include:

1. Formal chemical name. The Chemical Abstracts Service (CAS) or International Union of Pure and Applied Chemistry (IUPAC) name of the additive is acceptable.
2. Common names, synonyms, or trade names.
3. Chemical Abstracts Service (CAS) registry number. Providing CAS registry numbers for a petitioned food additive

and for impurities in and degradation products of the petitioned additive can help OFAS further identify the chemicals intended to be added to food. This facilitates the determination of exposure to and safety of the various chemicals present in food as the result of the use of the food additive. CAS registry numbers for new compounds and assistance with nomenclature can be obtained from CAS Client Services, Chemical Abstracts Service, P.O. Box 3012, Columbus, OH, 43210-0012.

4. Empirical and structural formulas and molecular or formula weights.
5. Composition of the food additive. For mixtures, identify as many of the components as feasible to reasonably define the composition of the mixture. In addition, information on the chemical composition and identity for each component in the mixture and a material balance should be provided.
6. For food additives of natural origin, information on the source (e.g., systematic name, genus, species, variability based on climate or other geographical factors).
7. Further characterizing information, such as data on chemical and physical properties of the food additive (e.g., melting point, boiling point, specific gravity, refractive index, optical rotation, pH, solubility, and reactivity) and chromatographic, spectroscopic, or spectrometric data (e.g., spectra from nuclear magnetic resonance, infrared, electronic absorption, or mass spectra) that can be used as a "fingerprint" for identification. If the particle size is important for the additive to achieve its intended technical effect, such that the additive is produced or processed using techniques or tools that manipulate the particle size and may contain altered particles that are formed as manufacturing byproducts, data on the size (average and distribution), shape, surface area (average and distribution), surface charge (zeta potential), and morphology of the particles, as well as any other size-dependent properties (e.g., agglomeration, aggregation, dispersion) should be included, as appropriate.

Specifications for identity and purity of the petitioned food additive should be proposed. If published specifications for the food additive are available, for example in the Food Chemicals Codex (FCC), 6th edition (2008) or current edition, then these should be cited and appropriately referenced. The data provided in this section of a food additive petition should represent a complete compositional analysis of the food additive, including:

1. A description of the food additive (e.g., physical form, odor, color, and solubility). For food additives derived from natural sources, the sources themselves should be clearly identified.
2. Identification tests for the food additive, including the method(s) used or reference(s) for a suitable method(s).
3. An assay of purity for the additive, including the method used or reference to a suitable method.
4. Physico-chemical characteristics of the food additive (e.g., ash content, moisture content, melting point, density, refractive index, pH).
5. Parameters related to the particle size, shape, and surface properties of the food additive, as appropriate, if particle size is important for the identity and functionality of the additive.
6. Limits for impurities and contaminants.
    a. A limit for lead should be proposed. In addition, limits for arsenic and heavy metals, such as cadmium and mercury, should be considered when their presence must be controlled.

b. Limits for any known natural toxicants or for microbial contaminants in or on a food additive derived from a natural source should be proposed.
c. Limits for residual reactants, reaction byproducts, and residual solvents should be proposed.

Conclusions

Dairy-based ingredients are emerging as popular food additives due to unique functionality associated with their addition. For approved ingredients or a GRAS substances, CFRs specifying identity and intended use govern their application over and above GMP, PMO, and Grade A HACCP requirements. For a new ingredient, an approval process is the most appropriate course of regulatory compliance. Dairy-based ingredients may either be GRAS approved or they may be approved through the new ingredient approval mechanism. While preparing submission documents, it is useful to adhere to advisory guidelines laid out by the FDA.

Note: Some of the information in this chapter has been derived from Chapter 22, Management Systems for Safety and Quality, published in *Dairy Processing and Quality Assurance* (Wiley-Blackwell, 2008).

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Chapter 16

Nutritive and Health Attributes of Dairy Ingredients

Ramesh C. Chandan

Introduction

Milk and milk products are considered to be functional foods, defined as foods containing significant levels of biologically active components that impart health benefits beyond basic nutrition. They are also referred to as wellness foods, healthful foods, or nutraceuticals. Functional foods play a significant role in the prevention of diseases such as cancer, coronary heart disease, atherosclerosis, stroke, diabetes, and liver ailments.

Various foods are provided with vital nutrients and health-related components when dairy ingredients are incorporated into them. This chapter provides basic information on the contribution of nutrients and health-enhancing attributes of commercially important dairy ingredients.

Milk and Milk Products as Functional Foods

Milk has been described as nature's nearly perfect food. It provides vital nutrients including proteins, essential fatty acids, minerals, and lactose in balanced proportions.

Definition of Milk

For commercial use in the United States, milk is defined as the whole, clean, lacteal secretion of one or more healthy cows properly fed and kept, excluding the milk obtained within 15 days before calving and three to five days afterward. Clearly, this excludes colostrum, the milk secreted immediately after the birth of a calf. It is interesting to acknowledge that colostrum is now an established functional food that contains high levels of antibodies to protect newborn calves.

Chemical Composition of Milk

From a chemical standpoint, milk is a complex fluid containing at least 100,000 separate molecules and chemical entities. In the dairy and food industry, the composition of milk and dairy ingredients is generally described in terms of its commercially significant constituents, namely, milk fat and nonfat solids (NFS) or milk-solids-not-fat (MSNF). The MSNF comprises of protein, lactose and minerals.

According to the U.S. Food and Drug Administration (FDA) standards of identity, milk "contains not less than 8.25% MSNF and not less than 3.25% milk fat." The major constituents of milk of mammals important in human nutrition are given in Table 16.1.

In terms of physical structure, milk is an opaque, white, heterogeneous fluid in which various constituents are held in multidispersed phases of emulsion, colloidal suspension, or solution (Table 16.2). The phase of the milk constituents influences their nutritional and physiological characteristics. The

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Table 16.1. Composition of milk commonly used for consumption in various regions of the world.

Animal	% Water	% Protein	% Fat	% Lactose	% Ash
Cow	87.3	3.4	3.7	4.8	0.7
Buffalo	82.7	3.6	7.4	5.5	0.8
Goat	87.7	2.9	4.9	4.1	0.8
Sheep	80.7	4.5	7.4	4.8	1.0
Horse	88.8	2.5	1.9	6.2	0.5
Camel	86.5	3.1	4.0	5.6	0.8

Adapted from Aneja et al. (2002), Fox (2003).
Source: Chandan and Kilara (2008)

Table 16.2. Physical state and particle size distribution in milk facilitating nutritional and physiological effects.

Physical state	Type of particles	Size, diameter (nm)
Emulsion	Fat globules	2,000–6,000
Colloidal dispersion	Casein-calcium phosphate	50–300
	Whey proteins	4–6
True solution	Lactose, salts, and other substances	0.5

Chandan and Kilara (2008)

larger surface area of the colloidal and emulsion state of proteins and lipids facilitates their digestion and absorption.

Role of Milk Constituents in the American Diet

Milk is comprised of unique nutrients that provide complete nutrition for the newborn (Figure 16.1). Most nutrition experts recognize milk and milk products as important constituents of a well-balanced and nutritionally adequate diet for children, adolescents, adults, and the elderly. In this regard, milk products complement and supplement nutrients available from grains, legumes, vegetables, fruits, meat, seafood, and poultry.

Milk and dairy products are considered to be nutrient-dense foods because they supply a high level of nutrients relative to their low caloric value. As shown in Table 16.3, dairy products contribute a significant proportion of nutrients in the American diet. The data in the table show that in 2005, milk and dairy products (other than butter) contributed just 7.6% of the total calories provided by all foods consumed. Concomitantly, milk and milk foods furnished 70.3% of the calcium, 30.1% of the phosphorus, 25% of the riboflavin, 15.7% of the vitamin A, 18.2% of the vitamin B_{12}, 18.1% of the protein, and 13.9% of the magnesium in the American diet. It is interesting to note that dairy products (other than butter) contribute relatively low levels of fat compared to the overall fat in the American diet. As the table shows, dairy products contributed just 8.3% of fat to the American diet. This may be ascribed to the fact that the dairy industry has responded to consumer demand by offering reduced fat, low-fat and nonfat dairy foods.

Nutrient Profile of Dairy Ingredients

Dairy ingredients furnish healthy constituents to food, including proteins, fat, lactose, and minerals. Milk proteins have a high nutritional value because of their high essential amino acid content. Thus, they complement and balance the relatively lower quality amino acid composition of many vegetable proteins in the human diet. They act independently and synergistically with each other. Apart from proteins, dairy ingredients contribute desirable minerals and vitamins to foods. The role of major and minor constituents in human nutrition is intertwined with newly discovered physiological benefits.

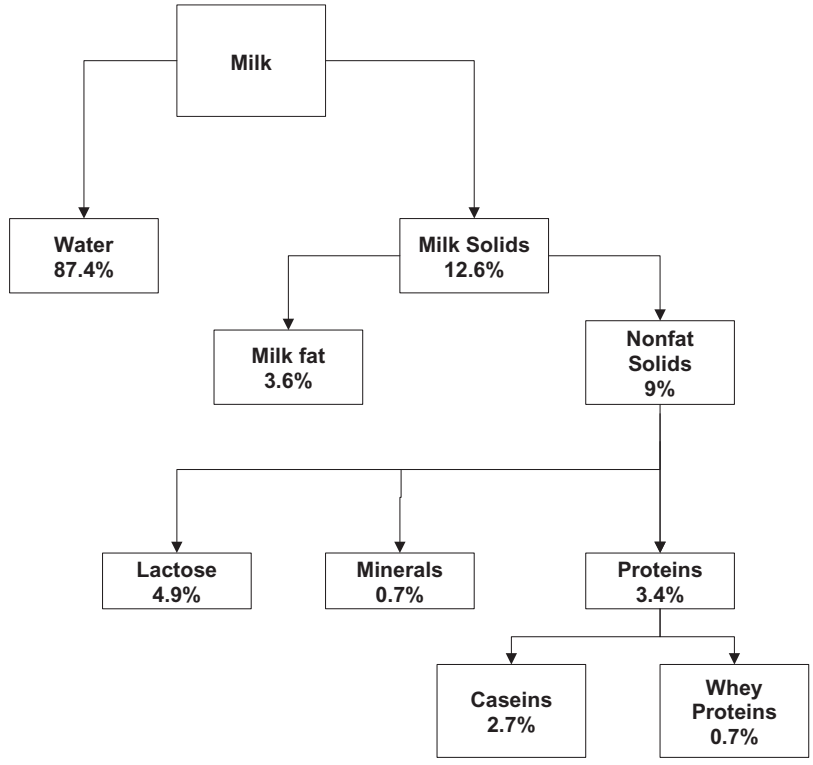

Figure 16.1. Major nutrient composition of milk. Chandan and Kilara (2008).

The nutrient content of fluid dairy ingredients is shown in Table 16.4. Each fluid dairy ingredient has a specific nutrient profile. Depending upon the level at which these ingredients are incorporated in a food, it is easy to calculate the contribution of energy, protein, fat, carbohydrate, minerals, and vitamins of a given dairy ingredient to the overall nutrient profile of the food in which they are incorporated. The nutrient profiles of concentrated dairy ingredients (sweetened condensed milk, nonfat dry milk, dry whole milk, dry acid whey, dry sweet whey, and dry buttermilk) are shown in Table 16.5. Data on cultured sour cream, unsalted butter, salted butter, and anhydrous butter oil are given in Table 16.6. Similar data for natural and processed cheeses are shown in Tables 16.7 and 16.8. The nutrient profile of selected unripened cheeses is given in Table 16.9.

Milk Proteins

Milk proteins constitute 38% of the SNF content of milk and 21% of the energy of whole milk. They are recognized as high-quality proteins, contributing 18.1% of the protein intake of the American diet.

Milk proteins contain all nine essential amino acids that the human body cannot synthesize and thus, must be furnished by the diet. Table 16.10 shows the recommended daily allowances for adults as well as the amino acids contributed by 2% reduced fat milk (Miller, et al., 2007). Both essential and non-essential amino acids are shown.

The quality of a protein is expressed in several ways. Milk protein and its fractions display outstanding nutritional quality as determined by different measurements. Table 16.11 shows the data to support this claim.

Table 16.3. Contribution of nutrients by dairy products (except butter) to the United States per capita food supply of nutrients in 2005.

Nutrient	Percent contribution
Energy	7.6
Fiber	0.4
Protein	18.1
Fat	8.3
Saturated fatty acids	16.6
Monounsaturated fatty acids	5.2
Polyunsaturated fatty acids	1.3
Cholesterol	12.5
Carbohydrate	4.3
Calcium	70.3
Phosphorus	30.1
Zinc	15.0
Copper	2.5
Magnesium	13.9
Potassium	16.0
Sodium	33.2
Iron	1.8
Selenium	9.8
Riboflavin	25.0
Vitamin A (RAE)	15.7
Vitamin B_{12}	18.2
Vitamin B_6	6.8
Folate	3.3
Thiamin	4.3
Ascorbic acid	2.5
Vitamin E	1.7
Niacin	1.1

Adapted from Hiza, Bente, and Fungwe (2008)

The major proteins of milk are casein and whey proteins in the ratio of 80:20. Casein further consists of various fractions including α_{S1}-casein and α_{S2}-casein, β-casein, and κ-casein (Table 16.12). Also shown are the major whey proteins of milk.

A number of proteins found in milk are now recognized for their physiological activity, including immunoglobulins, lactoperoxidase, lactoferrin, folate-binding protein, insulin-like growth factors (IGF-1 and IGF-2), mammary-derived growth factors (MDGF-I and MDGF-II), transforming growth factors ($TGF_{\alpha 1}$, $TGF_{\alpha 2}$, TGF_{β}), fibroblast growth factors, and platelet-derived growth factors. Peptides derived from milk proteins are gaining recognition for their biological and functional roles. For example, evidence points to physiologically active:

- Glycomacropeptides from κ-casein
- Phosphopeptides and caseinomorphins derived from caseins
- Immunomodulating peptides
- Platelet-modifying peptide
- Angiotensin-converting enzyme (ACE) inhibitor peptide (lowers blood pressure)
- Calmodulin-binding peptides
- Bactericidal peptides from lactotransferrin (Otter, 2003).

Both caseins and whey proteins of milk possess physiological and biological properties. The biological properties of milk proteins are summarized in Table 16.13.

Caseins

Caseins are divided into α_{s1}-, α_{s2}-, β-, and κ-fractions, which, along with the whey proteins β-lactoglobulin and α-lactalbumin, are gene-derived proteins synthesized in the mammary gland. All of these proteins are heterogeneous and exhibit genetic polymorphs. There are two to eight genetic variants differing from each other in one to 14 amino acids. The variants may have an impact on the protein concentration and functional properties of milk. The γ-fraction originates from β-casein by the effect of the native proteolytic enzyme of milk.

Caseins display distinctive structure, charge, physical, and biological properties as well as a nutritional role. The interaction of various caseins and calcium phosphate contributes to the formation of large colloidal complex particles called casein micelles. Hydrophobic interactions with calcium phosphate and sub-micelles seem to be involved in the formation of micelles. Micelle composition consists of 63% moisture and the dry matter consists of 92% to 94% protein and 6% to 8% colloidal calcium phosphate. Other associated salts are magnesium and citrate.

The caseins are phosphorylated proteins, containing one to 13 phosphoserine residues.

Table 16.4. Nutrient content of 100 g of fluid milk ingredients (without any additive).

Nutrients and units	Raw, whole fluid milk, 3.7% fat	Reduced fat, fluid milk, 2% fat	Low-fat, fluid milk, 1% fat	Nonfat (skim, fat-free) fluid milk	Cream, fluid, whipping	Buttermilk, fluid, cultured, low-fat	Yogurt, plain, low-fat
National Database no.	01078	01174	01175	01151	01053	01088	01117
Weight, g	100	100	100	100	100	100	100
% Moisture	87.69	89.21	89.92	90.84	57.71	90.13	85.07
Energy, kcal	66	50	42	34	345	40	63
Energy, kJ	268	210	177	142	1443	169	265
Protein, g	3.28	3.30	3.37	3.37	2.05	3.31	5.25
Fat, g	3.66	1.98	0.97	0.08	37.00	0.88	1.55
Saturated fatty acids, g	2.278	1.257	0.633	0.051	23.032	0.548	1.000
Monounsaturated fatty acids, g	1.057	0.557	0.277	0.021	10.686	0.254	0.426
Polyunsaturated fatty acids, g	0.136	0.075	0.035	0.003	1.374	0.033	0.044
Cholesterol, mg	14	8	5	2	137	4	6
Carbohydrate, g	4.65	4.8	4.99	5.09	2.79	4.79	7.04
Dietary fiber, g	0	0		0	0	0	0
Calcium, mg	119	120	125	122	65	116	183
Iron, mg	0.05	0.02	0.03	0.03	0.03	0.05	0.08
Magnesium, mg	13	11	11	11	7	11	17
Phosphorus, mg	93	92	95	101	62	89	144
Potassium, mg	151	140	150	156	75	151	234
Sodium, mg	49	47	44	42	38	105	70
Zinc, mg	0.38	0.48	0.42	0.42	0.23	0.42	0.89
Copper, mg	0.010	0.006	0.010	0.013	0.006	0.011	0.013
Manganese, mg	0.004	0.014	0.003	0.003	0.001	0.002	0.004
Selenium, µg	2.0	2.5	3.3	3.1	0.5	2.0	3.3
Vitamin C, mg	1.5	0.2	0.0	0.0	0.6	1.0	0.8
Thiamin, mg	0.038	0.039	0.020	0.045	0.022	0.034	0.044
Riboflavin, mg	0.161	0.185	0.185	0.182	0.110	0.154	0.214
Niacin, mg	0.084	0.092	0.093	0.094	0.039	0.058	0.114
Pantothenic acid, mg	0.313	0.356	0.361	0.357	0.255	0.275	0.591
Vitamin B_6, mg	0.042	0.038	0.037	0.037	0.026	0.034	0.049
Folate, total, µg	5	5	5	5	4	5	11

(continued)

Table 16.4. Nutrient content of 100 g of fluid milk ingredients (without any additive). *(cont.)*

Nutrients and units	Raw, whole fluid milk, 3.7% fat	Reduced fat, fluid milk, 2% fat	Low-fat, fluid milk, 1% fat	Nonfat (skim, fat-free) fluid milk	Cream, fluid, whipping	Buttermilk, fluid, cultured, low-fat	Yogurt, plain, low-fat
Folic acid, µg	0	0	0	0	0	0	0
Folate, food, µg	5	5	5	5	4	5	11
Vitamin B_{12}, µg	0.36	0.53	0.47	0.50	0.18	0.22	0.56
Vitamin A, RAE	33	28	14	2	411	14	14
Vitamin A, IU	138	102	47	15	1470	47	51
Vitamin D, IU	NA	1	1	0	27	1	0.0
Vitamin E, mg	NA	0.03	0.01	0.01	1.06	0.05	0.03
Vitamin K, µg	NA	0.2	0.1	0	3.2	0.1	0.2
Amino acids							
Tryptophan, g	0.046	0.040	0.040	0.040	0.029	0.036	0.030
Threonine, g	0.148	0.103	0.089	0.082	0.093	0.158	0.216
Isoleucine, g	0.198	0.183	0.187	0.150	0.124	0.204	0.286
Leucine, g	0.321	0.331	0.375	0.327	0.201	0.329	0.529
Lysine, g	0.260	0.233	0.287	0.252	0.163	0.277	0.471
Methionine, g	0.082	0.083	0.083	0.062	0.051	0.081	0.155
Cysteine, g	0.030	0.107	0.116	0.123	0.019	0.031	0.048
Phenylalanine, g	0.158	0.162	0.167	0.145	0.099	0.174	0.286
Tyrosine, g	0.158	0.153	0.142	0.148	0.099	0.139	0.265
Valine, g	0.220	0.218	0.217	0.180	0.137	0.243	0.434
Arginine, g	0.119	0.107	0.096	0.072	0.074	0.126	0.158
Histidine, g	0.089	0.073	0.084	0.075	0.056	0.095	0.130
Alanine, g	0.113	0.111	0.106	0.100	0.071	0.119	0.225
Aspartic acid, g	0.249	0.299	0.311	0.243	0.156	0.264	0.416
Glutamic acid, g	0.687	0.779	0.782	0.673	0.429	0.643	1.028
Glycine, g	0.069	0.061	0.063	0.050	0.043	0.073	0.127
Proline, g	0.318	0.368	0.359	0.343	0.199	0.334	0.622
Serine, g	0.178	0.199	0.208	0.169	0.111	0.172	0.325
Hydroxyproline, g	NA	0.000	0.000	0.000	NA	NA	NA

Calories factors: protein, 4.27; fat, 8.79; carbohydrate, 3.87
NA, not available
Adapted from USDA (2009)

Table 16.5. Nutrient content of 100 g of concentrated dairy ingredients.

Nutrients and units	Sweetened condensed milk	Nonfat dry milk	Dry whole milk	Dry acid whey	Dry sweet whey	Dry buttermilk
National Database no.	01095	01091	01212	01113	01115	01094
Weight, g	100	100	100	100	100	100
% Moisture	27.16	3.16	2.47	3.51	3.19	2.97
Energy, kcal	321	362	496	339	353	387
Energy, kJ	1342	1516	2075	1419	1476	1619
Protein, g	7.91	36.16	26.32	11.73	12.93	34.30
Fat, g	8.70	0.770	26.71	0.54	1.07	5.78
Saturated fatty acids, g	5.486	0.499	16.742	0.342	0.684	3.598
Monounsaturated fatty acids, g	2.427	0.201	7.924	0.149	0.297	1.669
Polyunsaturated fatty acids, g	0.337	0.030	0.665	0.021	0.034	0.215
Cholesterol, mg	34	20	97	3	6	69
Carbohydrate, g	54.40	51.98	38.42	73.45	74.46	49.00
Dietary fiber, g	0	0	0	0	0	0
Calcium, mg	284	1257	912	2054	796	1184
Iron, mg	0.19	0.32	0.47	1.24	0.88	0.30
Magnesium, mg	26	110	85	199	176	110
Phosphorus, mg	253	968	776	1349	932	933
Potassium, mg	371	1794	1330	2289	2080	1592
Sodium, mg	127	535	371	968	1079	517
Zinc, mg	0.94	4.08	3.34	6.31	1.97	4.02
Copper, mg	0.015	0.041	0.080	0.050	0.070	0.111
Manganese, mg	0.006	0.020	0.040	0.015	0.009	0.023
Selenium, µg	14.8	27.3	16.3	27.3	27.2	20.3
Vitamin C, mg	2.6	6.8	8.6	0.9	1.5	5.7
Thiamin, mg	0.090	0.415	0.283	0.045	0.519	0.392
Riboflavin, mg	0.416	1.550	1.205	2.060	2.208	1.579
Niacin, mg	0.210	0.951	0.646	1.160	1.258	0.876
Pantothenic acid, mg	0.750	3.568	2.271	5.632	5.620	3.170
Vitamin B_6, mg	0.051	0.361	0.302	0.620	0.584	0.338
Folate, total, µg	11	50	37	33	12	47
Folic acid, µg	0	0	0	0	0	0
Folate, food, µg	11	50	37	33	12	47
Vitamin B_{12}, µg	0.44	4.03	3.25	2.50	2.37	3.82
Vitamin A, RAE	74	6	258	17	8	49
Vitamin A, IU	267	22	934	59	30	175
Vitamin D, IU	6	0	0.5	0	0	0.5
Vitamin E, mg	0.16	0	0.58	0	0.02	0.10
Vitamin K, µg	0.6	0.1	2.2	0	0.01	0.4
Amino acids						
Tryptophan, g	0.112	0.510	0.371	0.241	0.205	0.484
Threonine, g	0.357	1.632	1.188	0.590	0.817	1.548
Isoleucine, g	0.479	2.188	1.592	0.581	0.719	2.075
Leucine, g	0.775	3.542	2.578	1.116	1.186	3.360
Lysine, g	0.627	2.868	2.087	1.008	1.030	2.720
Methionine, g	0.198	0.907	0.660	0.221	0.241	0.860
Cysteine, g	0.073	0.334	0.243	0.211	0.253	0.317
Phenylalanine, g	0.382	1.746	1.271	0.386	0.407	1.656
Tyrosine, g	0.382	1.746	1.271	0.300	0.363	1.656
Valine, g	0.529	2.420	1.762	0.579	0.697	2.296
Arginine, g	0.286	1.309	0.953	0.327	0.375	1.242
Histidine, g	0.214	0.981	0.714	0.230	0.237	0.930
Alanine, g	0.273	1.247	0.908	0.506	0.598	1.183
Aspartic acid, g	0.600	2.743	1.997	1.149	1.269	2.602
Glutamic acid, g	1.656	7.572	5.512	2.096	2.248	7.183
Glycine, g	0.167	0.765	0.557	0.211	0.280	0.726
Proline, g	0.766	3.503	2.549	0.699	0.786	3.322
Serine, g	0.430	1.967	1.432	0.541	0.622	1.866
Hydroxyproline, g	NA	NA	NA	0.000	NA	NA

Calories factors: protein, 4.27; fat, 8.79; carbohydrate, 3.87.
NA, not available
Adapted from USDA (2009)

Table 16.6. Nutrient content of 100 g of sour (cultured) cream, butter and butter products used as ingredients.

Nutrients and units	Sour cream, cultured	Butter, unsalted	Butter, salted	Butter oil, anhydrous
National Database no.	01056	01145	01001	01003
Weight, g	100	100	100	100
% Moisture	74.46	17.94	15.87	0.24
Energy, kcal	193	717	717	876
Energy, kJ	809	2999	2999	3664
Protein, g	2.07	0.85	0.85	0.28
Fat, g	19.73	81.11	81.11	99.48
Saturated fatty acids, g	11.507	51.368	51.368	61.024
Monounsaturated fatty acids, g	5.068	21.021	21.021	28.732
Polyunsaturated fatty acids, g	0.84	3.043	3.043	3.694
Cholesterol, mg	52	215	215	256
Carbohydrate, g	2.88	0.06	0.06	0
Dietary fiber, g	0	0	0	0
Calcium, mg	110	24	24	4
Iron, mg	0.17	0.02	0.02	0
Magnesium, mg	10	2	2	0
Phosphorus, mg	115	24	24	3
Potassium, mg	141	24	24	5
Sodium, mg	80	11	576	2
Zinc, mg	0.38	0.09	0.02	0.01
Copper, mg	0.019	0.016	0	0.001
Manganese, mg	0.011	0.004	0	0
Selenium, µg	2.6	1.0	1.0	0
Vitamin C, mg	0.9	0	0	0
Thiamin, mg	0.036	0.005	0.005	0.001
Riboflavin, mg	0.172	0.034	0.034	0.005
Niacin, mg	0.109	0.042	0.042	0.003
Pantothenic acid, mg	0.336	0.110	0.110	0.10
Vitamin B_6, mg	0.057	0.003	0.003	0.001
Folate, total, µg	7	3	3	0
Folic acid, µg	0	0	0	0
Folate, food, µg	7	3	3	0
Vitamin B_{12}, µg	0.28	0.17	0.17	0.01
Vitamin A, RAE	176	684	684	840
Vitamin A, IU	623	2499	2499	3069
Vitamin D, IU	1.4	60	60	73
Vitamin E, mg	0.44	2.32	2.32	2.80
Vitamin K, µg	1.8	7.0	7.0	8.6
Amino acids				
Tryptophan, g	0.035	0.012	0.012	0.004
Threonine, g	0.120	0.038	0.038	0.013
Isoleucine, g	0.138	0.051	0.051	0.017
Leucine, g	0.273	0.083	0.083	0.027
Lysine, g	0.233	0.067	0.067	0.022
Methionine, g	0.066	0.021	0.021	0.007
Cysteine, g	0.025	0.008	0.008	0.003
Phenylalanine, g	0.133	0.041	0.041	0.014
Tyrosine, g	0.129	0.041	0.041	0.014
Valine, g	0.165	0.057	0.057	0.019
Arginine, g	0.099	0.031	0.031	0.010
Histidine, g	0.078	0.023	0.023	0.008
Alanine, g	0.098	0.029	0.029	0.010
Aspartic acid, g	0.222	0.064	0.064	0.021
Glutamic acid, g	0.570	0.178	0.178	0.059
Glycine, g	0.064	0.018	0.018	0.006
Proline, g	0.262	0.082	0.082	0.027
Serine, g	0.161	0.046	0.046	0.015
Hydroxyproline, g	NA	NA	NA	NA

Calories factors: protein, 4.27; fat, 8.79; carbohydrate, 3.87
NA, not available
Adapted from USDA (2009)

Table 16.7. Nutrient content of 100 g of natural cheeses used as ingredients.

Nutrients and units	Cheddar	Cheddar/ colby, low-fat	Colby	Monterrey	Swiss	Mozzarella, part-skim, low-moisture	Blue
National Database no.	01009	01168	01011	01025	01040	01029	01004
Weight, g	100	100	100	100	100	100	100
% Moisture	36.75	63.10	38.20	41.01	37.12	46.46	42.41
Energy, kcal	403	173	394	373	380	302	353
Energy, kJ	1684	724	1647	1562	1591	1262	1477
Protein, g	24.90	24.35	23.76	24.48	26.93	25.96	21.40
Fat, g	33.14	7.00	32.11	30.28	27.80	20.03	28.74
Saturated fatty acids, g	21.092	4.342	20.218	19.066	17.779	10.877	18.669
Monounsaturated fatty acids, g	9.391	2.082	9.280	8.751	7.274	4.85	7.778
Polyunsaturated fatty acids, g	0.942	0.222	0.953	0.899	0.972	0.508	0.800
Cholesterol, mg	105	21	95	89	92	54	75
Carbohydrate, g	1.28	1.91	2.57	0.68	5.38	3.83	2.34
Dietary fiber, g	0	0	0	0	0	0	0
Calcium, mg	721	415	685	746	791	731	528
Iron, mg	0.68	0.42	0.76	0.72	0.20	0.25	0.31
Magnesium, mg	28	16	26	27	38	26	23
Phosphorus, mg	512	484	457	444	567	524	387
Potassium, mg	98	66	127	81	77	95	256
Sodium, mg	621	612	604	536	192	528	1395
Zinc, mg	3.11	1.82	3.070	3.00	4.36	3.13	2.66
Copper, mg	0.031	0.021	0.042	0.032	0.043	0.027	0.040
Manganese, mg	0.010	0.006	0.012	0.011	0.005	0.011	0.009
Selenium, µg	13.9	14.5	14.5	14.5	18.2	16.3	14.5
Vitamin C, mg	0	0	0	0	0	0	0
Thiamin, mg	0.027	0.012	0.015	0.015	0.063	0.101	0.029
Riboflavin, mg	0.375	0.221	0.375	0.390	0.296	0.329	0.382
Niacin, mg	0.080	0.051	0.093	0.093	0.092	0.119	1.016
Pantothenic acid, mg	0.413	0.183	0.210	0.210	0.429	0.090	1.729
Vitamin B_6, mg	0.074	0.045	0.079	0.079	0.083	0.079	0.166
Folate, total, µg	18	11	18	18	6	10	36
Folic acid, µg	0	0	0	0	0	0	0
Folate, food, µg	18	11	18	18	6	10	36
Vitamin B_{12}, µg	0.083	0.49	0.83	0.83	3.34	2.31	1.22
Vitamin A, RAE	265	60	264	198	220	160	198
Vitamin A, IU	1002	207	994	769	830	605	763
Vitamin D, IU	24	5	24	22	20	15	21
Vitamin E, mg	0.29	0.06	0.28	0.26	0.38	0.37	0.25
Vitamin K, µg	2.8	0.6	2.7	2.5	2.2	1.3	2.4
Amino acids							
Tryptophan, g	0.320	0.286	0.305	0.315	0.401	0.603	0.312
Threonine, g	0.886	0.798	0.845	0.871	1.038	0.151	0.785
Isoleucine, g	1.546	1.389	1.475	1.519	1.537	1.329	1.124
Leucine, g	2.385	2.145	2.275	2.344	2.965	2.138	1.919
Lysine, g	2.072	1.866	1.978	2.037	2.585	1.130	1.852
Methionine, g	0.652	0.588	0.622	0.641	0.784	0.603	0.584
Cysteine, g	0.125	0.111	0.119	0.123	0.290	0.135	0.107
Phenylalanine, g	1.311	1.179	1.251	1.289	1.662	1.184	1.087
Tyrosine, g	1.202	1.080	1.147	1.182	1.693	1.222	1.295
Valine, g	1.663	1.496	1.586	1.635	2.139	1.548	1.556
Arginine, g	0.941	0.847	0.898	0.925	0.927	0.603	0.711
Histidine, g	0.874	0.786	0.834	0.859	1.065	0.603	0.758
Alanine, g	0.703	0.633	0.670	0.691	0.914	0.828	0.644
Aspartic acid, g	1.600	1.439	1.527	1.573	1.569	1.912	1.436
Glutamic acid, g	6.092	5.480	5.813	5.990	5.704	5.220	5.179
Glycine, g	0.429	0.385	0.410	0.422	0.508	0.603	0.406
Proline, g	2.809	2.526	2.678	2.759	3.690	2.753	2.100
Serine, g	1.456	1.309	1.389	1.431	1.640	0.860	1.120
Hydroxyproline, g	NA	NA	NA	NA	NA	NA	NA

Calories factors: protein, 4.27; fat, 8.79; carbohydrate, 3.87.
NA, not available
Adapted from USDA (2009)

Table 16.8. Nutrient content of 100 g of process cheese and process cheese products used as ingredients.

Nutrients and units	Pasteurized process American cheese*	Pasteurized process American cheese, low-fat	Pasteurized process American cheese food*	Pasteurized process American cheese spread*	Cheese sauce	Cold pack American cheese food
National Database no.	01042	43275	01149	01150	01164	01045
Weight, g	100	100	100	100	100	100
% Moisture	39.16	58.90	43.15	47.65	66.86	43.12
Energy, kcal	375	180	328	290	197	331
Energy, kJ	1571	753	1373	1215	824	1386
Protein, g	22.15	24.60	19.61	16.41	10.33	19.66
Fat, g	31.25	7.00	24.60	21.23	14.92	24.46
Saturated fatty acids, g	19.694	4.410	15.443	13.327	8.034	15.355
Monounsaturated fatty acids, g	8.951	2.005	7.206	6.219	4.735	7.165
Polyunsaturated fatty acids, g	0.990	0.222	0.723	0.634	1.397	0.719
Cholesterol, mg	94	35	64	55	38	64
Carbohydrate, g	1.60	3.50	7.29		5.48	8.32
Dietary fiber, g	0	0	0	0	0	0
Calcium, mg	552	684	574	562	311	497
Iron, mg	0.19	0.43	0.84	0.33	0.35	0.84
Magnesium, mg	27	24	31	29	19	30
Phosphorus, mg	513	827	754	875	229	400
Potassium, mg	169	180	279	242	142	363
Sodium, mg	1489	1430	1596	1625	493	966
Zinc, mg	2.84	3.32	2.99	2.59	1.26	3.01
Copper, mg	0.016	0.033	0.030	0.033	0.019	0.030
Manganese, mg	0.008	NA	0.010	0.020	0.040	0.010
Selenium, µg	14.4	16.6	16.0	11.3	6.6	16.2
Vitamin C, mg	0	0	0	0	0.6	0
Thiamin, mg	0.027	0.030	0.029	0.048	0.044	0.030
Riboflavin, mg	0.353	0.390	0.442	0.431	0.243	0.446
Niacin, mg	0.069	0.080	0.140	0.131	0.204	0.074

Pantothenic acid, mg	0.482	NA	0.558	0.686	0.233	0.977
Vitamin B_6, mg	0.071	0.080	0.141	0.117	0.045	0.141
Folate, total, µg	8	9	7	7	10	5
Folic acid, µg	0	0	0	0	2	00
Folate, food, µg	8	9	7	7	8	5
Vitamin B_{12}, µg	0.70	0.77	1.12	0.40	0.35	1.28
Vitamin A, RAE	250	57	201	NA	82	159
Vitamin A, IU	945	215	762	788	312	705
Vitamin D, IU	23	5	NA	NA	8	NA
Vitamin E, mg	0.27	0.27	0.22	NA	0.09	NA
Vitamin K, µg	2.6	2.7	NA	NA	0.9	NA
Amino acids						
Tryptophan, g	0.323	NA	0.286	0.239	0.123	0.287
Threonine, g	0.719	NA	0.637	0.628	0.355	0.638
Isoleucine, g	1.024	NA	0.907	0.833	0.578	0.909
Leucine, g	1.958	NA	1.733	1.780	0.902	1.738
Lysine, g	2.198	NA	1.946	1.507	0.772	1.951
Methionine, g	0.573	NA	0.507	0.538	0.243	0.509
Cysteine, g	0.142	NA	0.126	0.105	0.056	0.126
Phenylalanine, g	1.125	NA	0.996	0.931	0.484	0.999
Tyrosine, g	1.212	NA	1.073	0.890	0.452	1.076
Valine, g	1.326	NA	1.174	1.366	0.627	1.177
Arginine, g	0.927	NA	0.821	0.545	0.351	0.823
Histidine, g	0.903	NA	0.799	0.509	0.311	0.801
Alanine, g	0.555	NA	0.491	0.602	0.279	0.493
Aspartic acid, g	1.361	NA	1.205	1.103	0.628	1.208
Glutamic acid, g	4.597	NA	4.070	3.475	2.214	4.080
Glycine, g	0.365	NA	0.323	0.311	0.170	0.324
Proline, g	2.253	NA	1.995	2.320	1.021	2.000
Serine, g	1.069	NA	0.946	1.037	0.539	0.949
Hydroxyproline, g	NA	NA	NA	NA	NA	NA

Calories factors: protein, 4.27; fat, 8.79; carbohydrate, 3.87.
* with disodium phosphate
NA, not available
Adapted from USDA (2009)

Table 16.9. Nutrient content of 100 g of unripened fresh cheeses used as ingredients.

Nutrients and units	Cottage cheese, creamed, 4% fat	Cottage cheese, low-fat, 1% fat	Cream cheese	Cream cheese, low-fat	Ricotta, whole milk	Ricotta, part-skim
National Database no.	01012	01016	01017	43274	01036	01037
Weight, g	100	100	100	100	100	100
% Moisture	79.79	82.48	54.44	66.72	71.70	74.41
Energy, kcal	98	72	342	201	174	138
Energy, kJ	412	303	1431	843	728	578
Protein, g	11.12	12.39	5.93	7.85	11.26	11.39
Fat, g	4.30	1.02	34.24	15.28	12.98	7.91
Saturated fatty acids, g	1.718	0.645	19.292	9.098	8.295	4.927
Monounsaturated fatty acids, g	0.778	0.291	8.62	3.996	3.627	2.314
Polyunsaturated fatty acids, g	0.123	0.031	1.437	0.658	0.385	0.260
Cholesterol, mg	17	4	110	54	51	31
Carbohydrate, g	3.38	2.72	4.07	8.13	3.04	5.14
Dietary fiber, g	0	0	0	0	0	0
Calcium, mg	83	61	98	148	207	272
Iron, mg	0.03	0.14	0.38	0.17	0.38	0.44
Magnesium, mg	8	5	9	8	11	15
Phosphorus, mg	159	134	106	152	158	183
Potassium, mg	104	86	138	247	105	125
Sodium, mg	364	406	321	470	84	125
Zinc, mg	0.40	0.38	0.51	0.57	1.16	1.34
Copper, mg	0.029	0.028	0.019	0.032	0.021	0.034
Manganese, mg	0.002	0.003	0.011	0.011	0.006	0.010
Selenium, µg	9.7	9.0	2.4	4.0	14.5	16.7
Vitamin C, mg	0	0	0	0	0	0
Thiamin, mg	0.027	0.021	0.020	0.040	0.013	0.021
Riboflavin, mg	0.163	0.165	0.125	0.185	0.195	0.185
Niacin, mg	0.099	0.128	0.145	0.125	0.104	0.078
Pantothenic acid, mg	0.557	0.215	0.570	0.845	0.213	0.242
Vitamin B_6, mg	0.046	0.168	0.035	0.045	0.043	0.020
Folate, total, µg	12	12	11	19	12	13
Folic acid, µg	0	0	0	0	0	0
Folate, food, µg	12	12	11	19	12	13
Vitamin B_{12}, µg	0.43	0.63	0.25	0.92	0.34	0.29
Vitamin A, RAE	37	11	366	161	120	107
Vitamin A, IU	140	41	1343	552	445	384
Vitamin D, IU	3	0	25	11	10	6
Vitamin E, mg	0.08	0.01	0.29	0.27	0.11	0.07
Vitamin K, µg	0	0.1	2.9	1.1	1.1	0.7
Amino acids						
Tryptophan, g	0.147	0.138	0.069	0.091	0.125	0.127
Threonine, g	0.500	0.550	0.233	0.308	0.517	0.523
Isoleucine, g	0.591	0.728	0.324	0.429	0.589	0.596
Leucine, g	1.116	1.274	0.657	0.869	1.221	1.235
Lysine, g	0.934	1.002	0.567	0.750	1.338	1.353
Methionine, g	0.269	0.373	0.191	0.252	0.281	0.284
Cysteine, g	0.066	0.115	0.042	0.055	0.099	0.100
Phenylalanine, g	0.577	0.668	0.291	0.384	0.556	0.562
Tyrosine, g	0.604	0.660	0.303	0.401	0.589	0.596
Valine, g	0.748	0.767	0.395	0.522	0.692	0.700
Arginine, g	0.497	0.565	0.235	0.311	0.632	0.639
Histidine, g	0.326	0.412	0.175	0.232	0.459	0.464
Alanine, g	0.384	0.643	0.184	0.243	0.499	0.505
Aspartic acid, g	0.905	0.839	0.514	0.680	0.995	1.007
Glutamic acid, g	2.603	2.684	1.304	1.725	2.446	2.474
Glycine, g	0.222	0.270	0.142	0.187	0.295	0.298
Proline, g	1.229	1.435	0.665	0.880	1.066	1.078
Serine, g	0.639	0.695	0.374	0.495	0.575	0.582
Hydroxyproline, g	NA	NA	0.000	0.000	NA	NA

Calories factors: protein, 4.27; fat, 8.79; carbohydrate; 3.87.
NA, not available
Adapted from USDA (2009)

Table 16.10. Distribution of amino acids in 2% milk and selected cheeses as compared to the recommended daily allowance for adults.

Amino acids	Concentration in 2% reduced fat milk, (mg/serving of 244 g)	Milk-solids not-fat[a] (mg/g)	Cheddar cheese-mg/oz (28 g)	Pasteurized Process American cheese, mg/oz (28 g)	Cottage cheese, mg/4 oz (113 g)	Recommended daily allowance for adults, (mg/day)[b]
Essential						
Histidine	230	10.31	248	256	516	980
Isoleucine	517	22.98	438	290	913	1,300
Leucine	835	37.16	676	555	597	2,900
Lysine	676	30.12	587	623	1,255	2,700
Methionine[c]	213	9.5	185	162	467	1,300
Phenyl alanine[d]	412	18.34	372	319	837	2,300
Threonine	385	17.12	251	204	688	1,400
Tryptophan	120	5.34	91	92	173	350
Valine	571	25.39	471	376	962	1,680
Non-essential						
Alanine	294	13.1	199	157	806	—
Arginine	309	13.76	267	263	709	—
Aspartic acid	647	28.79	454	386	1,051	—
Cysteine	78	3.5	35	40	144	—
Glutamic acid	1,786	79.48	1,727	1,303	3,363	—
Glycine	181	8.04	122	103	338	—
Proline	826	36.78	796	639	1,799	—
Serine	463	20.66	413	303	871	—
Tyrosine	412	18.34	341	344	827	—

[a]based on 8.67% MSNF for whole milk;
[b]values calculated for 70-kg male adult;
[c]total sulphur-containing amino acids, methionine plus cysteine;
[d]total aromatic amino acids, phenylalanine plus tyrosine
Adapted from Miller et al. (2007)

Table 16.11. Comparative nutritional measures of the quality of various food proteins.

Protein	PER	AAS	BV	PD	PDCAAS	NPU
Milk protein	3.1	1.27	91	95	1.21	86.45
Casein	2.5	1.24	77	100	1.23	76
Whey protein	3.2	1.16	104	100	1.15	92
Whole egg	3.9	1.21	100	98	1.18	94
Soy protein conc.	1.7	0.96	—	95	0.91	—
Wheat flour	0.6	0.38	61	91	0.42	56
Rice, polished	2.2	0.66	64	—	—	59

PER(protein efficiency ratio): Gain in body weight divided by weight of protein consumed by growing rats fed 10% (w/w) of test or reference protein
AAS (amino acid score): Content of the first limiting essential amino acid in test protein compared with the content of that essential amino acid in a reference pattern of essential amino acids
BV (biological value): Proportion of absorbed protein that is retained for body maintenance and growth
PD (protein digestibility): Proportion of food protein absorbed
PDCAAS (protein digestibility corrected amino acid score): Ratio of mg of limiting amino acid in 1 g of test protein and mg of the same amino acid in the reference requirement pattern multiplied with true digestibility
True Digestibility: I (F-f)/I, where I = nitrogen intake, F = total nitrogen excretion, and f = fecal nitrogen excretion on a protein-free diet
NPU (net protein utilization): Proportion of protein intake that is retained, calculated as BV × PD
Adapted from Schaafssma and Steijns(2000), Southward, (2002), Miller, et al, (2007). Source: Chandan and Kilara (2008)

Table 16.12. Major proteins, their fractions and polypeptides of cow's milk.

Casein fractions	Concentration (g/100 mL)
α_{s1}-casein	1.2–1.5
α_{s2}-casein	0.3–0.4
β-casein	0.9–1.1
κ-casein	0.3–0.4
Casein fragments (including γ-casein)	0.2–0.35
Total casein	2.4–2.8
Whey protein fractions	
β-lactoglobulin	0.2–0.4
α-lactalbumin	0.1–0.17
Immunoglobulins	0.05–0.18
Bovine serum albumin	0.02–0.04
Total whey protein	0.5–0.7

Adapted from Schaafsma and Steijns (2000), Chandan (2007).
Source: Chandan and Kilara (2008)

κ-casein exists in as many as nine glycosylated forms. It contains two cysteine molecules/molecule. As a result of disulfide bond formation, it can exist as polymers of two to eight units. Similarly, α_{S-2}–casein also contains two cysteines and exists in a dimeric form.

Casein micelles contain α_{s1}-, α_{S2-}-, β-, and κ-casein in the ratio of 3 : 1 : 3 : 1. Most of the α_{s1}- and α_{S2}- fractions and β-casein are located in the interior of the micelles, with κ-casein predominantly wrapped around the surface of the micelle. Casein fractions in the interior of the micelle are sensitive to calcium and become insoluble in its presence. However, κ-casein is not sensitive to calcium and thereby keeps the micelles containing calcium-sensitive caseins intact and suspended in the aqueous phase. κ-casein is a protein with hydrophilic carbohydrate moiety (sialic acid) that extends into the aqueous phase, further stabilizing the micelle. Casein micelles are stable under most heating, homogenization, and other dairy processing conditions.

Caseins have a certain distinctive amino acid makeup that impacts their processing and functional properties. They are rich in apolar and hydrophobic amino acids, namely valine, leucine, isoleucine, phenylalanine, tyrosine, and proline. The apolar amino acids normally are insoluble in water, but their nature is balanced by phosphate groups so that caseins exhibit some solubility.

Methionine and cysteine, the sulfur-containing amino acids, are relatively low in

Table 16.13. Bioactivity of milk proteins.

Protein	Physiological effect
Caseins	Precursors of bioactive peptides; calcium and phosphorus carrier. Bioactive peptides of caseins such as casomorphins lower gut motility and gastric emptying rate, enhance uptake of amino acids by intestinal epithelial cells, and enhance immune response and phagocytic activity Casokinins (ACE-I) enhance blood flow to intestinal epithelium Isracidin, casecidins, and kappacin exhibit bactericidal activity against pathogenic organisms. κ-casein enhances the growth of bifidobacteria in the gastrointestinal tract
Whey proteins	Confer passive immunity for disease prevention, reduce risk of heart disease and lower blood pressure, anti-viral and anti-cancer activity, control of gut microflora, control of cellular glutathione level
β-lactoglobulin	Binds zinc, calcium, and fat-soluble vitamins The branched chain amino acids enhance the immune system
α-lactalbumin	Lactose synthesis in mammary gland, anti-carcinogenic and immune enhancing effects May be associated with stress reduction, increase serotonin production in brain, improve mood, and decrease cortisol level
Immunoglobulins A, M, G^1, G^2	Antibodies against diarrhea and GI tract disturbances Support passive immune function
Bovine serum albumin	Antioxidant and anti-mutagenic Binds free fatty acids and pro-oxidant transition metals
Lactoferrin	Bacterial anti-toxin binding, antibacterial, anti-viral, immune modulating, anti-inflammatory, anti-thrombic activity, anti-carcinogenic, antioxidant, iron absorption Enhances proliferation of intestinal epithelial cells, humoral immune response, and growth of bifidobacteria in gastrointestinal tract
Lactoperoxidase	Antimicrobial, antioxidant, immune enhancing properties
Lysozyme	Antimicrobial, synergistic with immunoglobulins and lactoferrin

Adapted from Chandan (1999), Gobbetti, et al. (2007), Harper (2000), Hoolihan (2004)

caseins, which impacts their nutritional deficiency. In contrast, the content of the essential amino acid lysine is high. In the human diet, the high lysine content is helpful in complementing and balancing the low-lysine plant proteins. The ε-amino group of lysine present in caseins interacts with the aldehyde group of lactose at elevated temperatures, leading to the formation of brown pigments. This also explains browning of heat-sterilized milk and nonfat dry milk during extended storage. The high proline content of caseins results in low α-helix and β-sheet in their secondary structure, giving them the ability for more proteolytic degradation and enhanced digestion (Otter, 2003).

Among the minor milk caseins, γ-casein is the C-terminal fragment of β-casein, a product of attack by the natural proteolytic enzyme plasmin. The N-terminal residue is the proteose-peptone fraction. These hydrolysis products of β-casein occur at a range of 3% to 10% of the total casein content of milk. The stage of lactation and health status of the cow affect their concentration.

Biologically Active Peptides Derived from Casein

Peptides derived from caseins are biologically active and display significant extra nutritional attributes for maintaining normalcy of physiological functions in human subjects.

Table 16.14 lists bio-active peptides originating from caseins.

Table 16.14. Physiological attributes of bioactive peptides derived from digestion of caseins and whey proteins.

Peptide	Origin	Function
β-casomorphin-4	β-casein	Opioid agonist, induces muscular rigidity
β-casomorphin-4amide	β-casein	Opioid agonist, most potent casomorphin
β-casomorphin-5	β-casein	Increases pain threshold, causes behavioral change
β-casomorphin-7	β-casein	Increases prolactin release (in rat)
β-casomorphin-11	β-casein	*In vivo* digestion product
Casoxin-A and B	κ-casein	Opioid anatagonistic
Casoxin-C	κ-casein	Opioid agonist, phagocyte-stimulating activity
Casokinin-5 and 6	α_{s1}-casein	ACE inhibitory, anti-hypertensive
Casoplatelins	κ-casein	Antithrombotic
Casocidin-1	α- and β-caseins	Antimicrobial
Immunopeptides	α- and β-caseins	Immunostimulants
Phosphopeptides	α- and β-caseins	Mineral carriers
Glycomacropeptide	κ-casein	Suppress appetite, anti-platelet, anticancer, antihypertensive, prevent dental caries, gingivitis, antiviral, antibacterial, bifidogenic/probiotic
β-Lactorphin	β-lactoglobulin	Opioid agonist, inhibition of
α-lactorphin	α-lactalbumin	angiotensin-I-converting enzyme
Lactkinins	β-lactoglobulin α-lactalbumin	ACE inhibitory (antihypertensive)
Lactoferricin	Lactoferrin	Antimicrobial activity
Lactoferroxins	Lactoferrin	Opioid antagonist

Adapted from Aimutis (2004), Boehm et al. (2007), Harper (2000), Pihlanto-Leppalo (2003), Saxelin et al. (2003)

Bioactive peptides are generated during digestive processes in the body and during the fermentation processes used in fermented dairy foods (Gobbetti et al., 2007). These peptides are inactive in the native proteins but assume activity after they are released from them. They contain three to 64 amino acids and display largely hydrophobic character and are resistant to hydrolysis in the gastrointestinal tract. They can be absorbed in intact form to exert various physiological effects locally in the gut or they may have a systemic effect after entry into the circulatory system. Casomorphins derived from milk caseins are known to be opioid agonists, while casoxins act as opioid antagonists. The opioids have analgesic properties similar to opium. Casokinins are antihypertensive (lower blood pressure), casoplatelins are antithrombotic (reduce blood clotting), and phosphopeptides are mineral carriers.

Casein phosphopeptides may aid in bioavailability of calcium, phosphorus, and magnesium for optimum bone health; may help prevent dental caries, and may play a role in secretion of entero-hormones and immune enhancement. Scientific evidence suggests a role of casein peptides in blood pressure regulation. Conversion of angiotensin-I to angiotensin-II is inhibited by certain hydrolyzates of casein and whey proteins. Angiotensin-II raises blood pressure by constricting blood vessels; thus, its inhibition lowers blood pressure. This ACE inhibitory activity therefore makes dairy foods a natural functional food for controlling hypertension. A commercial ingredient derived by the hydrolysis of milk protein has an anxiolytic bioactive peptide with anti-stress effects. Psychometric tests and measurement of specific hormonal markers have displayed an anti-stress effect.

The glycomacropeptide released from κ-casein as a result of proteolysis may be involved in regulating digestion as well as modulating platelet function and thrombosis in a beneficial way. It is reported to suppress appetite by stimulating the CCK hormone. Consequently, it may be a significant ingredient of satiety diets designed for weight reduction. Furthermore, this peptide may inhibit binding of toxin in the gastrointestinal tract.

Some miscellaneous bioactive factors are being discovered. Specific proteins for binding vitamin B_{12}, folic acid, and riboflavin may assist in enhancing bioavailablity from milk and other foods. A fat globule membrane protein called butyrophilin is a part of the immune system. Other growth factors in milk may help gut repair after radiation or chemotherapy.

Whey Proteins

Whey proteins, also called serum proteins, provide an excellent balance of essential amino acids. The amino acid profile resembles that of skeletal muscle, making them effective in stimulating protein synthesis in adult muscle. Thus, they preserve muscle mass and enhance health. Whey proteins also enhance fat loss. Whey proteins contain more branched-chain amino acids than any other protein, which are metabolized (to generate energy) in the muscle rather than in the liver. This property makes them suitable for use by endurance athletes such as marathon runners. In general, whey proteins enhance humoral immune response. The sulfhydryl-containing amino acids cysteine and glutathione are related to immune response. The branched-chain amino acids stimulate muscle glutamine synthesis, which is involved in immune function. Glutathione formation is facilitated by the high cysteine content of whey proteins, which in turn controls significant antioxidant defenses and immune function in the body.

Whey proteins are especially rich in cysteine. β-lactoglobulin contains 33 mg of cysteine/g protein, while α-lactalbumin and bovine serum albumin contain 68 and 69 mg cysteine/g protein, respectively. The –SH compounds are also involved in quenching toxic free-radicals.

In sports nutrition, the high content of arginine and lysine amino acids of whey proteins may help in stimulating release of growth hormones, leading to an increase in muscle mass and a decline in body fat. Furthermore, glutamine protects the immune system from decline caused by overtraining.

Whey proteins consist of β-lactoglobulin and α-lactalbumin, bovine serum albumin, immunoglobulins (mainly IgG1, IgG2, and IgM), lactoferrin, proteose-peptone, and a number of diverse enzymes.

β-Lactoglobulin

β-Lactoglobulin, a major whey protein of milk, displays the presence of four genetic variants. In addition to the two genetic variants A and B, variants C and D also have been reported. β-lactoglobulin is rich in sulphur amino acids, containing five cysteine residues. It exists as a dimer linked by 1–3 disulfide bonds. β-lactoglobulin is a fairly heat labile protein. It stimulates lipolysis and thereby generates rancidity. It also acts as a carrier of vitamin A. The large numbers of lysine residues can result in lactosylation and accompanying changes in the physical properties of the protein.

α-Lactalbumin

α-lactalbumin is a major protein in human milk, but in cow's milk it is second in preponderance to β-lactoglobulin. Three genetic variants are reported, but the milk of western cows only contains variant B. This protein is rich in tryptophan and the sulphur amino acids cysteine and methionine. There are four disulfides in the molecule, and it exists as a monomer. α-lactalbumin has 54 amino acid linkages identical with the enzyme lysozyme. It is a glycoprotein as well as a metalloprotein. One mole of calcium is bound to each protein molecule, which confers heat stability on α-lactalbumin. This protein has a physiological role in the synthesis of lactose in the mammary gland. It is a component of lactose synthetase along with uridine diphosphate-galactosyl transferase, catalyzing the transfer of galactose to glucose to form lactose.

α-lactalbumin is a calcium-binding protein, thereby enhancing calcium absorption.

It is an excellent source of essential amino acids such as tryptophan and cysteine. Tryptophan regulates appetite, sleep-waking rhythm, and pain perception, and cysteine is important in functions of –SH compounds.

Immunoglobulins

There are five major classes of immunologlobulins: IgA, IgD, IgE, IgG, and IgM. Their concentration is very high (100 g/L) in the first two to three milkings after the birth of a calf birth, but their concentration falls to 0.6 to 1 g/L soon after. Immunoglobulins are antibodies synthesized in response to stimulation by specific antigens. These offer nonspecific humoral response to Gram-negative enteric and aerobic bacteria. Accordingly, they provide passive immune protection to the newly born calf. The basic structure of all immunoglobulins is similar: two identical light chains (23,000 Da) and two identical heavy chains (53,000 Da) joined by disulfide bonds. The complete molecule has a molecular weight of about 180,000 Da. The antigenic sites are located at the $–NH_2$ terminal of the respective chain. Of the five immunoglobulin classes, IgG is the predominant fraction of milk, comprising about 90% of the total colostral immunoglobulins. Relatively smaller concentrations of IgM and IgA also are present in progressively decreasing amounts.

The immunoglobulins of milk are important for imparting immune defense for the host. IgG1 is a major component. Other fractions are IgG2, IgA, and IgM, all of which provide passive immunity.

A number of colostrum products are marketed as functional ingredients for foods. Colostrum contains several functional constituents, including antibodies, lactoferrin, lactoperoxidase, cytokines, and growth factors. The antibodies act as antimicrobial agents against infection from rotavirus (which causes diarrhea), *Escherichia coli* (which causes food poisoning), *Candida albicans* (which causes yeast infections), *Streptococcus mutans* (which causes dental caries), *Clostridium difficile* (which causes antibiotic-associated diarrhea), *Cryptosporium parvum* (which causes food poisoning), and *Helicobacter pylori* (which causes ulcers and gastritis). Colostrum stimulates the active immune system by enhancing the activity of natural killer cells and phagocytes. Colostrum powder is manufactured by a special drying process to ensure activity. Milk protein concentrate prepared from the milk of hyperimmunized cows is said to relieve the joint pain of arthritis by complementing the body's natural anti-inflammatory substances.

Bovine Serum Albumin

As the name indicates, bovine serum albumin originates from blood. It spills into milk during synthesis in the udder. It is a large molecule with a binding ability for fatty acids and metals.

Lactoferrin

Lactoferrin, also known as lactotransferrin, is a glycoprotein that displays a strong tendency to bind ionic iron due to the presence of two metal binding sites. The average lactoferrin content for cow milk is 0.32 mg/ml. The molecular weight of lactoferrin varies between 73,700 and 74,000 Da. Lactoferrin displays a very strong chelating tendency for ionic iron and forms a salmon-red pigment. It is a single peptide chain with two lobes, each of which is capable of binding iron. The iron-free form of lactoferrin is known as apolactotransferrin, which is colorless. Lactoferrin displays activity against several Gram-positive and Gram-negative bacteria, yeasts, fungi, and viruses. It shows a particularly strong inhibitory effect toward Gram-negative enteropathogenic bacteria by virtue of its ability to bind free ionic iron, which is essentially required for the growth of enteropathogenic microorganisms. Thus, lactoferrin plays a role in non-specific defense of the

host against invading pathogens. In addition to the antibacterial effect in the gut of the calf, a nutritional role in iron metabolism has also been ascribed to lactoferrin. Its iron-binding characteristic aids in enhancing iron absorption. It stimulates and protects cells involved in the host defense mechanism by controlling cytokine response.

Biologically Active Peptides from Whey Proteins

As shown in Table 16.14, a number of peptides derived from whey proteins exert physiological activity. The bioactive peptides of whey proteins have been shown to positively influence body composition, satiety, and weight management. In addition, the bioactive peptides are ACE-inhibitory.

Milk Enzymes

Milk is a repository of a variety of enzymes; more than 60 indigenous enzymes have been reported in cow's milk. They are either associated with the milk fat globule membrane (xanthine oxidase, sulfhydryl oxidase, and γ-glutamyltransferase), skim milk serum (catalase, superoxide dismutase), or micelles of casein (plasmin and lipoprotein lipase).

Lactoperoxidase breaks down hydrogen peroxide and exerts an antibacterial effect. Therefore, it is considered to be a natural preservative. Lysozyme displays antimicrobial activity against Gram-positive bacteria and acts by lysing cell walls. Lysozyme may indirectly affect the defense systems as an immunomodulator through the stimulation of immune system by breaking down products of the cell wall (peptidoglucan). The nutritional role of the milk enzymes is questionable because they are destroyed by heat treatment in milk processing.

Milk Fat

Many foods contain lipids in varying degree. Table 16.15 shows the fat contribution from major food groups in the U.S. food supply. Clearly, each food groups provides fat in our diet. However, most of the fat intake in 2005 was attributed to shortening (25.6%); salad, cooking, and edible oils (25.1%); and meat, poultry, and fish (23.3%). Food groups such as fruits, vegetables, legumes, and grains contribute minor levels of fat to our total fat intake. Most nutritionists recommend that daily fat intake not exceed 30% of all calories consumed, which amounts to a daily consumption of a maximum of approximately 68 g of fat in a 2,000-calorie diet.

Table 16.15. Percentage of contribution of fat from major food groups in the U.S. food supply from 2001 to 2005.

Food group	2001	2002	2003	2004	2005
Butter	2.6	2.4	2.5	2.5	2.4
Margarine	4.1	3.5	2.9	2.9	2.1
Shortening	23.5	22.6	22.3	22.6	25.6
Lard and beef tallow	3.0	3.2	3.4	3.3	3.5
Salad, cooking, and other edible oils	25.6	26.9	27.3	27.7	25.1
Meat, poultry, and fish	20.4	21.7	21.6	20.2	23.3
Eggs	2.1	2.0	2.0	2.0	1.9
Legumes, nuts, and soy	3.7	3.5	3.8	4.0	3.5
Grain products	2.4	2.2	2.3	2.3	2.2
Fruits	0.5	0.5	0.5	0.6	0.5
Vegetables	0.5	0.4	0.4	0.5	0.4
Sugars and sweeteners	0	0	0	0	0
Miscellaneous	1.1	1.0	1.0	1.1	1.1

Adapted from Hiza, Bente, and Fungwe (2008)

Food Lipids and Cardiovascular Disease

By definition, cardiovascular disease (CVD) includes diseases and injuries of the blood vessels (arteries and veins) of the heart, entire body and brain. Stroke, which involves cessation of blood flow in the brain, is a form of CVD. As the population ages, the incidence of CVD and the corresponding financial impact continue to escalate. Research into CVD has accelerated in the last 50 years, and the studies have elucidated, to a degree, how nutrition and genetics impact heart health.

Contribution of Food Lipids to Blood Lipid Profile

Fat plays a vital role in life as an important source of energy as well as a depot of energy storage. Fat is a concentrated source of energy (8.79 kcal/g). It protects organs and insulates the body from environmental temperature effects, is a critical component of cell membranes, assists in the regulation of cellular traffic, influences the action of insulin, and is involved in the boy's inflammation status.

Cholesterol is a fraction of the dietary and blood lipid profile. It comes from dietary fat but is predominantly synthesized in the liver. Cholesterol is important in its role as a precursor of hormones (estrogen, testosterone) and vitamin D.

Many studies have supported a positive association between risk of CVD and elevated blood cholesterol, some of its fractions, and triglycerides. Blood lipid-protein agglomerates (lipoproteins) are indicative of the risk.

Low-density lipoproteins (LDL-C) carry cholesterol from the liver to various parts of the body, and the cells extract fat and cholesterol for their needs. However, excessive levels of LDL-C in the blood leads to deposits (plaques) of wax-like material in the walls of coronary and other arteries. The plaques result in narrowing of coronary arteries, thereby constricting blood flow. Eventually the flow of blood (and oxygen) to the heart is interrupted, causing heart attack. Deprived of vital oxygen in a section of the heart, the heart tissue dies. If a plaque breaks loose and is lodged in the brain, it may cut off blood supply to a section of the brain, causing a stroke. Therefore, it is desirable to control the level of LDL-C in blood to preserve blood flow.

High-density lipoproteins (HDL-C) constitute a protective fraction of blood lipids. They remove cholesterol from the bloodstream, LDL-C, and walls of arteries. HDL-C transports cholesterol back to the liver.

Triglycerides originate from dietary fats and circulate in the bloodstream. They perform an important function by transporting fat to the cells. However, excessive amounts of blood triglyceride are involved in plaque formation and are not healthy.

In general, elevated LDL-C in blood (higher than 100 mg/dl) and a high level of blood triglycerides (higher than 150 mg/dl) are believed to have a positive correlation with CVD. The HDL-C protects an individual from CVD. An optimum level of HDL is higher than 60 mg/dl.

Although, these guidelines are still commonly used, some modifications are being suggested. Westman (2009) has advocated "a change from dietary fat restriction (which lowers LDL) to dietary carbohydrate restriction (which lowers triglycerides and raises HDL)".

The American Heart Association (AHA) (Lichtenstein et al., 2006) has recommended goals for CVD risk reduction:

- Consume an overall healthy diet
- Aim for a healthy body weight (body-mass index of 18.5 to 24.9 kg/m^2).
- Aim for optimal levels of blood LDL-C and HDL-C and triglycerides
- Aim for normal blood pressure, which is below 120 mm Hg, systolic, and below 80 mm Hg, diastolic. Pre-hypertension is systolic 120 to 139 and diastolic 80 to

90 mm Hg. Hypertension is systolic, above 140, and diastolic, above 90 mm Hg
- Aim for a normal glucose level in the blood
- Be physically active (exercise regularly)
- Avoid use of and exposure to tobacco products

To achieve these goals, the 2006 recommendations of the American Heart Association (Lichtenstein et al., 2006) are:

- Balance calorie intake and physical activity to achieve/maintain a healthy body weight
- Consume a diet rich in vegetables and fruits
- Choose whole grain, high-fiber foods
- Consume fish, especially oily fish, at least twice a week
- Limit the intake of saturated fat to less than 7% of energy, trans fat to less than 1% energy, and cholesterol to 300 mg/day by:
  - — Choosing lean meats and vegetable alternates
  - — Selecting skim milk, 1% fat milk, and low-fat dairy foods. The goals include one cup of low-fat milk (244 g), one cup of low-fat yogurt (227 g), and 1.5 oz (42 g) of cheese/day. The contribution of milk fat from the dairy foods should not exceed 13–16 g per day. This corresponds to 6–7% of fat calories in a 2,000-calorie diet.
  - — Minimizing intake of partially hydrogenated fats
- Minimize intake of beverages and foods with added sugars
- Choose and prepare foods with little or no salt
- If alcohol is consumed, do so in moderation
- When eating food that is prepared outside the home, follow the AHA diet and lifestyle recommendations

Physiological Role of Milk Fat

Milk fat exists in an emulsion form in milk, making it highly digestible. In terms of CVD, the functional properties of all dietary lipids are attributed to the fatty acid makeup. The chemical structure of dietary fatty acids is implicated in their ability to raise or lower serum cholesterol. Milk fat is comprised primarily of triglycerides of fatty acids, which make up 95% to 96% of milk fat. The remaining milk fat is comprised of diglycerides, monoglycerides, free fatty acids, phospholipids, cholesterol, and other nutrients (Table 16.16).

Milk fat is a concentrated form of energy. It is responsible for 49% of the total energy of whole milk, 35% of the energy of 2% fat-reduced milk, and 21% of the energy of 1% low-fat milk. Fat protects organs and insulates body from environmental temperature effects. Anhydrous milk fat (100 g) contributes 3,818 IU (939 RE) of vitamin A, 30 to 90 IU of vitamin D, 3 mg of vitamin E, and 0.1 mg of vitamin K (Aneja

Table 16.16. Constituents of bovine milk lipids.

Lipid fraction	g/L milk	% Weight
Triacylglycerols/triglycerides	30.7	95.80
Diacylglycerols/diglycerides	0.72	2.30
Monoacylglycerols/monglycerides	0.03	0.08
Free fatty acids	0.09	0.28
Phospholipids	0.36	1.11
Cholesterol	0.15	0.46
Cholesterol esters	0.006	0.02
Total	32.056	100.05

Chandan (2007).
Source: Chandan and Kilara (2008)

Table 16.17. Fatty acid composition of bovine milk fat.

Type	Number of carbon atoms*	Systematic name	Common name	% Weight in milk	% Weight in butter
Saturated fatty acids	4:00	Butanoic acid	Butyric acid	4.5	5.31
	6:00	Hexanoic acid	Caproic acid	2.3	2.81
	8:00	Octanoic acid	Caprylic acid	1.3	1.56
	10:00	Decanoic acid	Capric acid	2.7	3.14
	12:00	Dodecanoic acid	Lauric acid	3.0	3.39
	14:00	Tetradecanoic acid	Myristic acid	10.6	10.78
	16:00	Hexadecanoic acid	Palmitic acid	28.2	28.13
	18:00	Octadecanoic acid	Stearic acid	12.6	10.62
	20:00	Eicosanoic acid	Arachidic acid	0.20	0.20
Monounsaturated fatty acids	14:1	Tetradecenoic acid	Myristoleic acid	0.9	0.9
	16:1	Hexadecenoic acid	Palmitoleic acid	1.8	1.38
	18:1	Octadecenoic acid	Oleic acid	21.4	20.84
	20:1	Eicosenoic acid	Gadoleic acid	0.6	0.29
	22:1	Docosenoic acid	Erucic acid	—	0.09
Polyunsaturated fatty acids	18:2	Octadecadienoic acid	Linoleic acid	3.3	2.72
	18:3	Octadecatrienoic acid	Linolenic acid	3.2	0.56
	18:3 n-3	—	α-linolenic acid	0.3	0.48
	18:3 n-6	—	γ-linolenic acid	2.9	0.08
	18:4 n3	Octadecattetraenoic acid	Parinaric acid	—	0.27
	20:2	Eicosadienoic acid	—	—	0.03
	20:3	Eicosatrienoic acid	—	—	0.10
	20:4 n6	Eicosatetraenoic acid	Arachidonic acid	0.2	0.14

*Number of carbon atoms, followed by number of unsaturated bonds in the molecule.
Adapted from: Jensen (1995)

et al., 2002). Vitamin A performs important roles in vision, body growth, reproduction, bone metabolism, and immune function. Vitamin D is essential for a healthy skeleton and is involved in absorption of calcium and phosphorus in the gastrointestinal tract. Vitamin E works as an antioxidant that protects cell membranes and lipoproteins from oxidative deterioration by free radicals. It also stimulates the immune system and helps maintain cell integrity. Vitamin K is vital for blood clotting function and may play a role in bone protection.

Milk fat also is a source of essential fatty acids and linolenic acids. Essential fatty acids are not synthesized by human body in required amounts, thus, they must be supplied by the diet. Arachidonic acid with four double bonds is present in traces. Its precursor is linoleic acid. Omega-3-linoleic acid and its products EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), although present in trace amounts, exert a significant physiological role.

The physiological attributes of milk fat are attributed to its fatty acid make-up. More than 400 distinct fatty acids have been detected in milk. Table 16.17 shows the fatty acid composition of the fat of milk and butter (Jensen, 1995).

Typically, the fat in milk consists of 65% saturated, 23% monounsaturated, and 10% polyunsaturated fatty acids. Because of fractionation during the butter-making process, the fat of butter consists of 66% saturated, 23% monounsaturated and 4% polyunsaturated fatty acids.

The roles of the various fatty acids and other milk fat constituents in human health are discussed below.

Short and intermediate chain saturated fatty acids. Milk fat is distinctive in that it has short-chain and intermediate-chain saturated fatty acids (C_4 to C_{12}), accounting for 11% to 12% of its total fatty acids. These water-soluble fatty acids are preferentially liberated by lipase. Butyric acid liberated by the hydrolysis of milk fat is absorbed intact through the intestinal wall. Caproic, caprylic, and capric acids are then transported to the liver via the portal vein and are used for quick energy. This function is important in early life and is helpful in athletic and other activities requiring quick bursts of energy. It also is a favorable factor in milk fat digestion throughout life. Short-chain and intermediate-chain fatty acids are not implicated in raising the plasma cholesterol level.

Long-chain saturated fatty acids. Worldwide, general public dietary recommendations call for restricting the intake of saturated fat to control CVD. However, there are conflicting data regarding the simple relationship between dairy consumption and CVD (German et al., 2009). Previously it was thought that all saturated fatty acids enhance the serum cholesterol level. However, it has been discovered that the blood cholesterol level is affected, depending on the nature of the individual saturated fatty acid. The chain length and physical state of the fatty acid determine its atherogenic effect. It seems that most long-chain saturated fatty acids, with the exception of stearic acid, raise the LDL-C. Stearic acid (10% to 12% level in milk fat) is neutral in this regard (Miller et al., 2007). Palmitic and myristic acids, as free fatty acids, are primarily implicated in inducing cholesterol increase.

The positional location of individual fatty acids in the triglycerides is also relevant to CVD. In fact, the *sn*-2 positions on the glycerol molecule are mainly occupied by myristic ($C_{14:0}$), palmitic ($_{C16:0}$), and oleic acids ($C_{18:1}$). Because lipases involved in digestion display positional specificity in hydrolyzing fatty acids from the *sn*-1 and *sn*-3 positions in the triglyceride molecule, it is likely that digested fat contains only a proportion of the total palmitic and myristic acids in the form of absorbable free fatty acids. The saturated fatty acids left intact as part of monoglycerides may not be bioavailable. Thus, the available fatty acid composition of digested fat is not necessarily synonymous with the chemical composition of the fat.

A recent review concluded that there is no clear evidence that dairy food consumption is consistently associated with a higher risk of CVD (German et al., 2009). Nonetheless, it is prudent to balance intake of saturated fat in the diet with vegetable oils containing unsaturated and polyunsaturated fatty acids.

Mono- and polyunsaturated fatty acids. Milk fat contains approximately 27% of mono- and polyunsaturated fatty acids. Their consumption is considered to be cardioprotective because they lower LDL-C.

Essential fatty acids. Essential fatty acids (EFAs) are not synthesized by the human body in the required amounts, so they must be supplied by the diet. Milk fat furnishes EFAs. Arachidonic acid ($C_{20:4}$), with four double bonds, is present at the 0.1% to 1% level. Its precursor is linoleic acid. Omega-3-linoleic acid and its products, eicosapentaenoic acid ($C_{20:5}$; EPA) and docosahexaenoic acid ($C_{22:6}$; DHA), exert a significant physiological role even though they are only present in trace amounts. These acids are anti-inflammatory. They are beneficial in preventing heart disease; improving mental function and vision; and reducing the risk of colon, prostate, and breast cancers. By including fish oil, algae, flax seeds, sesame seeds, nuts, soybean oil, and certain grains (rye), it is possible to balance the intake of omega fatty acids in the diet.

Conjugated linoleic acids. Conjugated linoleic acids (CLA) are a class of fatty acids found in animal products such as milk fat. In fact, milk fat is the richest source of CLA (2.4 to 28.1 mg/g of fat). Rumen flora synthesizes CLA. The CLA isomer found in dairy

products is predominantly the most biologically active CLA, identified as *cis*-9, trans-11-18:2 isomer. CLAs have been demonstrated to exhibit potent physiological properties. They are a strong antioxidant constituent of milk fat and may prevent stomach, colon, and breast cancers. CLA have been shown to enhance the immune response and reduce the risk of heart disease by reducing prostaglandin PGE-2 levels, which otherwise promote inflammation, artery constriction, and blood clotting. Studies have indicated that CLAs may help in weight reduction, increasing bone density, reducing chronic inflammation, and normalizing blood glucose levels by increasing insulin sensitivity.

Trans-fatty acids. Trans-fatty acids (TFAs) are generated from unsaturated fatty acids by the hydrogenation process in vegetable oils. The hydrogenation process reduces the unsaturation level, thereby conferring shelf life stability. Concomitantly, hydrogenation increases the risk of CVD. Trans-fats raise the LDL-C and simultaneously lower the protective HDL-C. In this context, the main trans-fatty acid is identified as elaidic acid, 18:1, trans-9.

The milk of cows and other ruminants contain natural TFAs. Milk fat contains TFAs at the 3.56% level, but their chemical structure and physiological effects are opposite to the TFAs from hydrogenated vegetable oils. Several studies have shown that TFAs from milk fat do not increase the risk of CVD. The TFAs of ruminant milk are trans-vaccenic acid (18 : 1, trans-11) and rumenic acid (18:2 cis-9 trans-11). The naturally occurring milk TFAs are classified as CLAs, and as enumerated above, they possess potent beneficial effects.

Phospholipids. Phospholipids are present in the range of 2 to 10 mg/g of milk fat. They are beneficial in terms of CVD. Rich in polyunsaturated fatty acids, phospholipids are constituents of membranes. They play an important role in cell interaction with hormones, antibodies, and mineral ions. They aid in emulsification of lipids, thereby enhancing their absorption along with that of fat-soluble vitamins and carotenoids. Phospholipids are considered to protect gastric mucosa and may even extend protection from pathogenic microorganisms.

Sphingolipids are another constituent of milk fat; they occur at a level of only 0.6 mg/g. Recent studies show sphingolipids are hydrolyzed in the gastrointestinal tract to ceramides and sphingoid bases, which help in cell regulation and function. Experimental animal studies show that they inhibit colon cancer, reduce serum cholesterol, and elevate HDL-C, the protective cholesterol. They may also protect against bacterial toxins and infection.

Dietary cholesterol. It is now known that only a minor percentage (less than 25%) of the blood serum cholesterol arises from dietary cholesterol. The bulk of blood cholesterol is synthesized in the liver, which is controlled by the individual genetic makeup of an individual. The ability to absorb cholesterol from the gastrointestinal tract and its excretion in the feces varies widely among people. In general, the typical cholesterol content of milk fat is 3 to 4 mg/g.

Bovine milk fat globule membrane. The envelope surrounding the milk fat contains several health-promoting factors. Both protein and non-protein constituents are potential nutraceuticals. The phospholipid constituents of the fat globule membrane protect against colon cancer, gastrointestinal pathogenic organisms, Alzheimer's disease, stress-related diseases, and depression. Other membrane components also exhibit health properties, including inhibiting the growth of cancer cells, lowering serum cholesterol, inhibiting *Heliobacter pylori* (involved in stomach ulcers), and suppressing β-glucuronidase of the intestinal *Escherichia coli.* In addition, the membrane-situated enzyme xanthine oxidase acts as a bactericidal agent. Butyrophilin is a possible suppressor of multiple sclerosis.

Lactose

Lactose monohydrate, the major carbohydrate of milk, ranges from 4.8% to 5.2%. Lactose stimulates the absorption of calcium and magnesium. It has a relatively lower glycemic index of 46, as compared to 100 for glucose and 60 for sucrose. This makes lactose in skim milk suitable for diabetics and in weight control diets. It is less cariogenic than other sugars. Lactulose is a compound formed from lactose in heated milk products; heated milk contains up to 0.2% lactulose. Lactulose is not a digestible ingredient; therefore, it acts somewhat like a soluble fiber. Lactulose is generally used to treat constipation and chronic encephalopathy. Recent data indicate that lactulose may enhance calcium absorption in the intestine. It stimulates the growth of *Bifidobacterium bifidum* and is thereby beneficial in establishing useful microflora in the gut.

Certain ethnic groups in the United States, particularly a majority of African Americans, Asians, and Southern Europeans, experience symptoms of lactose intolerance, including bloating, flatulence, abdominal pain, and diarrhea, after consuming milk and milk products. Lactose intolerance is mainly attributed to discontinuation of milk consumption after infancy, as seen in the dietary pattern of such ethnic groups. In such cases, epithelial cells in the intestinal mucosa tend to lose the ability to secrete lactase enzyme due to absence of lactose in the diet. Lactase is a non-persistent enzyme in certain individuals, resulting in the distressing symptoms following milk intake. Lactase, or β-galactosidase, is a key enzyme in digestion of lactose. It catalyzes the hydrolysis of lactose to glucose and galactose, which are rapidly absorbed. Temporary absence of lactase from the digestive tract also may be exhibited during or following enteric infections, when the surface lining of intestinal mucosa is damaged due to invasion of enteropathogenic bacteria. Symptoms of lactose intolerance (maldigestion) may be seen when milk is consumed following weaning for several years or after enteric infection.

In the past, it was believed that lactose maldigesters should avoid milk and dairy products. However, new evidence shows that most non-persistent lactase individuals can tolerate two cups of milk spread over a day or a cup with meals. They also may choose lactose-reduced products or low-lactose products, or use lactase supplements. In the case of lactose malabsorption, the symptoms are ameliorated by consuming yogurt containing live and active cultures. Yogurt and fermented milks furnish the enzyme lactase to assist in digesting lactose. Lactose-reduced milk and ice cream products also are available. By consuming these products, lactose-maldigesters receive the vital nutrients of dairy foods, especially, calcium, protein, vitamins, and minerals.

Minerals

Milk is an excellent source of minerals (Table 16.18), including bioavailable calcium. Milk supplies assimilable calcium and phosphorus in an optimum ratio. The major source of dietary calcium is dairy products, supplying as much as 75% of the dietary intake in developed nations. The bioavailability of calcium is further enhanced by the presence of vitamin D, lactose, and phosphoprotein (casein). One of the primary functions of calcium is to provide strength and structural properties to bones and teeth. Lack of adequate calcium intake, particularly during the growth phase, leads to osteoporosis or brittle bones in later life.

Calcium is involved in muscle contraction (including heartbeat), blood coagulation, enzyme reactions, stimulation of hormonal secretions, and cell signaling. It is important in blood pressure control and it is a factor in the prevention of colon cancer. Phosphorus is also critical in bone mass formation, and takes part in various metabolic processes in

Table 16.18. Major and minor minerals of cow's milk.

Major minerals	Mean (mg/100 mL)	Range (mg/100 mL)
Calcium, total	121	114–130
Calcium, ionic	8	6–16
Citrate	181	171–198
Chloride	100	90–110
Magnesium	12	9–14
Phosphorus, inorganic	65	53–72
Potassium	144	116–176
Sodium	58	35–90
Trace Elements	**µg/100 g milk**	**µg/100 g milk**
Boron	27	—
Chromium	1	0.8–1.3
Cobalt	0.1	0.05–0.13
Copper	20	10–60
Fluoride	12	3–22
Iodine	26	—
Iron	45	30–60
Manganese	3	2–5
Molybdenum	7	2–12
Nickel	2.5	0–5
Selenium	12	5–67
Silicon	260	75–700
Zinc	390	200–600

Adapted from Fox (2003), Chandan (2007).
Source: Chandan and Kilara (2008)

the body. It is a crucial component of the genetic material DNA and RNA. Iron is essential in the formation of hemoglobulin and in cytochrome activity. A deficiency causes anemia. Iron is further involved in brain function, immunocompetence, and the synthesis of lipids.

Magnesium is also a part of bone mass, and it is involved in many metabolic pathways. Zinc is a component of several metabolic enzymes and DNA. It is involved in immune system functioning. Iodine is necessary for the formation of thyroid hormone, which regulates growth and metabolism. Copper is important in energy metabolism and as an antioxidant; it also is involved in collagen synthesis and iron utilization. Manganese is a cofactor of many metabolic enzymes. Chloride is an oxidizing agent and constitutes a vital ingredient of stomach acid.

Table 16.19. Proximate vitamin concentration of milk.

Vitamins	Concentration in 100 mL milk
A	40 µg RE
D	4 IU
E	100 µg
K	5 µg
B_1	45 µg
B_2	175 µg
Niacin	90 µg
B_6	50 µg
Pantothenic acid	350 µg
Biotin	3.5 µg
Folic acid	5.5 µg
B_{12}	0.45 µg
C	2 mg

Adapted from Chandan (2007).
Source: Chandan and Kilara (2008)

Potassium is a major electrolyte in blood and tissues and helps in blood pressure regulation in conjunction with sodium. Sodium is further involved in nerve conduction, active transport, and bone formation.

Vitamins and Other Minor Constituents

A balance of minerals and vitamins is required to promote health and well being. They must be supplied by food and supplements because they are not manufactured by the body. Milk contains both fat-soluble and water-soluble vitamins. Table 16.19 gives the concentration of fat-soluble vitamins A, D, E, and K; and water soluble vitamins B and C.

Natural vitamin A activity in milk is due to retinol and the pigment β-carotene. Their level as well as those of vitamin D and E varies in milk according to the season and feed profile. The diet's richest source of vitamin D is vitamin-D-fortified milk. Exposure to sunshine helps to activate this vitamin. Vitamin D assists in calcium absorption and in forming and maintaining strong bones. It is also recognized for its role in the prevention of the bone disease rickets. More recent research has shown that vitamin D reduces the risk of several types of cancer,

and improves immune function. It also protects against multiple sclerosis and helps to reduce falls in the frail elderly.

Vitamin E is an antioxidant. Vitamin K is present in milk but its dietary nutritional role is probably minor.

Milk is an important source of water-soluble dietary B vitamins. They are stable to the various heating and processing conditions to which milk is normally subjected. Vitamin B_1, thiamin, is a cofactor in carbohydrate metabolism. Vitamin B_2 is involved in the oxidation reactions of glucose, fatty acids, amino acids, and purines. Niacin facilitates utilization of carbohydrates, fat synthesis, and tissue respiration. Pantothenic acid participates in fatty acid metabolism. Vitamin B_6 is critical in protein metabolism. Folic acid acts as a growth factor and is involved in DNA synthesis. Vitamin B_{12} is required for growth, blood formation, and nerve tissue functioning. Biotin has a role in metabolism of carbohydrates, lipids, nucleic acid, and proteins. Ascorbic acid (vitamin C) is necessary for collagen formation, wound healing, and absorption of non-heme iron, and it provides resistance to infections. However, the vitamin C content of milk and dairy ingredients is relatively low.

Role of Milk Products in Nutrition for Different Age Groups

Milk and milk products contribute to health throughout human life. They ensure adequate intake of energy, fat, protein, lactose, vitamins, and minerals needed for children's growth and development. There is a consensus that infants should preferably receive only mother's milk for first six months of life. Infant formula can be substituted as warranted by certain circumstances. After six months, whole cow's milk can be introduced in small servings along with cereals, lean meats, eggs, fruits, vegetables, cereals, legumes, and other weaning foods. Iron must be supplemented in the diet. Low-fat milk can be given after two years of age, and nonfat milk should not be introduced until after 5 years of age. The 2005 dietary guidelines for Americans recommend three cups of nonfat or low-fat milk or equivalent nutrients derived from yogurt, cheese, and other dairy products per day for Americans nine years of age and older.

Intake of milk and dairy foods should be continued throughout life to prevent chronic diseases related to nutrient deficiency and to maintain good health. In the case of lactose malabsorption, it is possible to continue intake of dairy nutrients from lactose-free milk and other lactase-treated ice cream and low-lactose dairy products. Yogurt and culture-containing dairy foods are well tolerated by lactase-deficient individuals. A small percentage of individuals are allergic to cow's milk. They can derive required nutrients from goat's milk or soy milk.

The Role of Dairy Products in Disease Prevention and Management

Traditionally, the nutritional role of milk has been linked to the supply of essential and non-essential nutrients for optimal human growth, development, and sustenance. Now more emphasis is being placed on prevention of chronic diseases by dietary and lifestyle changes, including the role of specific dairy components that help to reduce the risks of chronic conditions. Dairy products can play a role in weight management, body fat loss, obesity, bone health and osteoporosis prevention, blood pressure reduction, type-2 diabetes, and combating certain cancers.

Weight Management

Epidemiologic evidence from one study indicates that the risk of weight gain was 67% lower in the group consuming the most dairy products as compared with the group consuming least dairy products (Huth et al., 2006). Human clinical trials with obese

subjects have indicated that during caloric reduction, there is a greater loss of body weight and body fat in diets containing adequate calcium from calcium supplements. Interestingly, this loss is relatively greater when the identical level of calcium is contributed by dairy sources. It appears that weight and fat loss by a calorie-controlled diet is caused by changes in metabolic partitioning of dietary energy, which is maximized by calcium intake (1,200 to 1,300 mg/day) from dairy products.

Another mode of calcium action in weight loss in a low-calorie diet may be interaction of calcium with dietary fat to form a soap-like material in the gut, which is not absorbed and is subsequently excreted. The distribution of body fat loss is also different in that a reduced-calorie diet alone resulted in a 19% loss of body fat around the trunk area; however, the loss around the trunk area was 50% in a diet containing calcium from supplemental sources and 66% in a diet containing dairy products (Zemel et al., 2005). It is important to note that the fat loss with added dairy products is conditional upon the restriction of total caloric intake. When the diet is high in calories, adding dairy products does not help in weight and fat loss. Accordingly, weight and fat loss should occur when three to four servings of dairy products are part of a low-calorie diet.

Type 2 Diabetes

Management of type-2 diabetes is critical for controlling the resulting complications, namely cardiovascular disease, renal failure, blindness, and amputations. Dietary intervention for controlling this disorder is the subject of research, and several studies have shown that lifestyle modifications, including diet, are important factors in preventing type-2 diabetes. Epidemiological studies have indicated that consuming high levels of dairy foods significantly reduces the risk of developing type-2 diabetes in men and women.

Conjugated linoleic acid (CLA), a natural component of milk fat, has been reported to improve the human body's poor regulation of insulin when administered for eight weeks or longer. It improves utilization of glucose, lowering its level in the blood of type-2 diabetic patients.

Bone Health and Prevention of Osteoporosis

Osteoporosis is related to progressive loss of bone tissue, which results in skeletal weakness and consequently leads to bone fractures after age 50. Optimum bone growth needs adequate amounts of dietary protein; vitamins A, C, D, and K; and calcium; phosphorus; magnesium; copper; manganese; zinc; and fluoride. These nutrients should be supplied by a variety of food sources. Dairy products are significant sources of calcium, phosphorus, and magnesium in the American diet.

Optimum bone development requires adequate dietary calcium to achieve peak bone mass. Bone is comprised of 50% protein and approximately 50% calcium phosphate crystals. The bone tissue process is in a dynamic state throughout life. The older bone breaks down and newer bone tissue constantly replaces it. During childhood and the teen years, provided that adequate nutrients are available, bone formation by far exceeds the breakdown (resorption) phase. Approximately 85% to 90% of peak bone mass is achieved by age 18 in girls and age 20 in boys. Bone mass in the early stages of life is a good determinant of bone strength later in life.

In adults, the rate of bone resorption is in equilibrium with that of bone formation. If bone resorption exceeds bone formation, a net loss of bone mass occurs, leading to bone porosity, fragility, and fractures. Certain types of bone cells involved in bone remodeling cease to function, resulting in progressive loss of bone mass. Patients with osteoporosis receive medications to activate remodeling

cells and are encouraged to consume calcium, vitamin D, and other dietary nutrients. The 2005 Dietary Guidelines recommend three cups of low-fat or nonfat milk or equivalent from dairy products for adults to help maintain bone health.

Osteoporosis occurs more commonly in women than men. Key elements in developing osteoporosis are genetic factors, age, physical activity, and nutrient intake. In women, osteoporosis is aggravated by menopause (loss of estrogen). Nevertheless, there is a consensus that maximizing peak bone mass by adequate calcium, phosphorus, and other minerals from dairy and food sources early in life is necessary for retaining higher bone mass and reducing the risk of bone fractures later in life.

Hypertension Control

Calcium and potassium are positively associated with regulation of blood pressure. Milk and dairy foods are good sources of these minerals and are now regarded as exerting an antihypertensive effect. Various population studies have shown the relationship between dietary intake of 1,000 mg/day or more and 40% to 50% reduction in the prevalence of hypertension. Randomized clinical trials have shown that calcium from food sources is more effective than calcium from supplements. The blood-pressure-lowering effect of dairy foods is not related solely to calcium content, but it is related to other minerals such as potassium and magnesium, vitamins, proteins, and essential fatty acids (Huth et al., 2006). In this regard, milk proteins are documented to be sources of bioactive peptides generated by proteolytic enzymes involved in digestive processes or by the action of lactic cultures used in fermented dairy products. These peptides lower blood pressure by ACE-inhibitor activity, as shown in animal and human trials to reduce blood pressure from the hypertensive range to normal levels (Wrick, 2007). The scientific evidence shows a beneficial role of including three servings of dairy foods in diets high in fruits and vegetables in preventing hypertension.

As discussed in previous sections, milk proteins are precursors of bioactive peptides that are capable of inhibiting the activity of the angiotensin-I-converting enzyme (ACE). This enzyme regulates blood pressure, electrolytes, and fluid balance in the body. ACE converts inactive angiotensin-I hormone into angiotensin-II, which constricts vascular smooth muscle, leading to elevation of blood pressure. Thus, an inhibitory effect of the peptide derived from milk protein lowers blood pressure. In this regard, whey protein hydrolyzates are particularly cited for their blood pressure lowering ability (Miller et al, 2007).

Cancer

The effect of diet on risk of cancer is significant: 30% of all cancers are related to diet. It is known that certain components of foods, when consumed excessively, may promote cancer; this includes calories, alcohol, and fat. On the other hand, some food components, including dairy, are preventative. Calcium and vitamin D are be beneficial against colon cancer. Dietary calcium normalizes hyperproliferation of colonic epithelium cells in individuals at risk of colon cancer. CLA and vacceinic acid found in all dairy foods confer desirable anti-carcinogenic and anti-atherogenic effects. CLA is also beneficial in glucose and fat metabolism in type-2 diabetic subjects. Because the fat content of popular dairy foods is low, their CLA content is also low. The isolated CLA is now being used as a supplement ingredient in foods.

Enhancement of Health Properties by Culturing of Milk

Fermented milks such as yogurt are enhanced functional foods because they contain the

nutrients of milk, live cultures, and products of the metabolic activities of starter microorganisms. They particularly contain live and active cultures in significant numbers to bestow physiological benefits to the consumer. Fundamentally, bacterial mass content and the products of the lactic fermentation distinguish yogurt from milk.

Probiotics and Beneficial Cultures are foods or supplements containing concentrates of defined strains of living microorganisms that, upon ingestion in certain doses, exert health benefits beyond inherent basic nutrition. Probiotics and associated ingredients may add an attractive dimension to cultured dairy foods by conferring special functional attributes.

Several hundred bacterial species inhabit the distal regions of the human digestive tract. Their population exceeds the total cell count in human body. Functions of the intestinal flora include modulation of cell growth and differentiation, antagonistic activity against pathogens and other infections, immune stimulation of gut-associated lymphoid tissue, reduction of blood lipids, and biosynthesis of vitamins. The colonies of diverse gut bacteria exist in equilibrium in healthy individuals. Factors such as stress, age, gastrointestinal disturbances, and antibiotic therapy are known to upset the balance of gut microflora and result in malfunctioning of their digestive and metabolic effects. Probiotics help restore the balance.

Milk is an excellent medium to carry or generate live and active cultured dairy products (Chandan and Shah, 2006; Shah, 2006; Vasilijevic and Shah, 2007). The buffering action of the milk proteins keeps the probiotics active during their transit through the gastrointestinal tract. In general, worldwide consumption of fermented milk products has increased due to their high nutritional profile, unique flavor, desirable texture, and remarkable safety against food-borne illness.

The cultures associated with health benefits are yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*), other lactobacilli, and bifidobacteria (Chandan and Shah, 2006). Yogurt organisms possess several documented health attributes. To bolster probiotic function, most commercial yogurt is generally supplemented with various species of *Lactobacilli* and *Bifidobacteria*. Yogurt starter bacteria, *Lb. delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*, are also thought to act as probiotics because of their health-promoting effects. In particular, yogurt bacteria have been scientifically demonstrated to assist in lactose digestion, reduce or prevent diarrhea episodes, and strengthen immune defenses of the host. They are reported to persist and remain viable throughout the human gastrointestinal tract. The continuous ingestion of live products ensures abundant numbers to maintain their functional status. Even with intestinal isolates such as *Lb. acidophilus*, it is necessary to dose regularly rather than to assume that a few doses allow the organisms to colonize the gut permanently. The reported health benefits (Pannell and Schoenfuss, 2007) associated with yogurt and probiotic cultures include:

- Stimulating the immune system
- Improving digestive regularity and alleviating constipation
- Reducing symptoms of lactose intolerance
- Reducing risk factors for colon cancer initiation
- Increasing calcium absorption
- Reducing the risk of cardiovascular disease by lowering serum cholesterol
- Alleviating inflammatory bowel disease and irritable bowel syndrome by restoring the normal balance of gastrointestinal microflora
- Enhancing resistance to colonization by pathogenic organisms

Table 16.20. Specific strains of probiotics and their documented benefits.

Strain	Benefits reported in human trials
L. acidophilus NCFM	Lactose digestion, reduction of bacterial overgrowth in small intestine
L. acidophilus(CUL60)+ B. bifidum (CUL20)	Reduction of fecal toxin of *C. difficile*
L. casei DN114-001	Enhanced immune function
L. casei Shirota YIT9029	Enhanced immune function, balanced intestinal microbiota, combating recurrence of superficial bladder cancer
L. helveticus R0052+L. rhamnosus R0011	Controlling diarrhea in children, eradicating *Heliobacter pylori* infection
L. johnsonii La1/Lj1	Enhanced immune function, eradication of *Heliobacter pylori* infection
L. plantarum 299V	Relief of irritable bowel syndrome, postsurgical gut nutrition
L. reuteri SD2112	Controlling diarrhea, improvement in immune function
L. reuteri RC14+L. rhamnosus R0011	Controlling diarrhea in children, eradicating *Heliobacter pylori* infection
L. rhamnosus GG	Prevention of infectious diarrhea in children and atopic dermatitis, enhancing immune function
L. salivarius UCC118	Alleviation of inflammatory bowel disease
B. animalis DN173-010	Normalizing intestine transit time
B. infantis 35624	Alleviation of irritable bowel syndrome
B. lactis BB-12	Enhancing immune function, alleviation of diarrhea in children
B. lactis HN019 (DR10)	Enhancing immune function in the elderly
B. longum BB536	Alleviation of allergy symptoms, balancing microbial ecology
VSL #3 (blend of S. thermophilus, four strains of lactobacillus, and three strains of bifidobacterium)	Alleviation of inflammatory bowel conditions
S. thermophilus (many strains)	Assistance in lactose digestion
Sachharomyces cerevisiae (boulardii) lyo	Alleviation of antibiotic-induced diarrhea and *C. difficile* infections.

Adapted from Sanders (2007)

- Reducing symptoms of eczema and skin diseases associated with the gut immune system

Because many health benefits of cultures are strain-specific, the recent trend is to use proven strains in dairy foods. Table 16.20 lists the documented health attributes of defined strains of probiotic cultures.

Yogurt and fermented milks are commonly supplemented with various functional ingredients. In addition to probiotics, they include prebiotics and fiber, plant sterol esters, omega-3 fatty acids, minerals, and vitamins.

Prebiotics and fiber include polysaccharides (inulin), oligosaccharides (fructooligosaccharides; FOS), galactooligosaccharides (GOS), and lactulose. They are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or limited number of bacteria in the colon that have the potential for improving the health of the host. They stimulate probiotic organisms such as bifidobacteria and lactobacilli. Prebiotics provide nutrients for colonic bacteria, thereby sustaining balance among various populations of enteric bacteria. Health attributes of prebiotics reported in the literature include prevention of colon cancer initiation, stimulation of immune response, providing a barrier against pathogenic organisms, prevention of adhesion of pathogens and toxins,

rendering systemic effects on blood lipids, and assistance in mineral absorption. In this aspect, their action resembles probiotics.

Note: Some of the information in this chapter has been derived from Chapter 18, Role of Milk and Dairy Foods in Nutrition and Health, published in *Dairy Processing and Quality Assurance* (Wiley-Blackwell, 2008).

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Chapter 17

Dairy Ingredients in Dairy Food Processing

Tonya C. Schoenfuss and Ramesh C. Chandan

Introduction

Milk-based ingredients are added to dairy products for their functional and nutritional benefits. The ingredients can be the primary ingredient or a functional additive. In either case, the functional properties of gelation, foaming, emulsification, and water binding are used. The ingredient source, processing history, and sensory characteristics of the ingredient are of great importance for product quality. Dairy ingredients can be incorporated into dry mixes as well as liquid, cultured, gelled, and frozen products to create tasty, nutritious products.

Shelf-stable Powdered Products

Dry dairy ingredients are added to beverages, dessert products, and other foods because of their flavor, nutritional properties, and ability to gel and create texture. Dairy ingredients in dry mixes must be flowable, easily reconstituted, and resistant to oxidative off-flavors and browning. Table 17.1 summarizes the steps and factors affecting the reconstitution of dairy powders. The ability of food processors and consumers to reconstitute dairy powders for their intended use greatly affects food processors' and consumers' opinions of a product. Likewise, the sensory properties of powdered dairy ingredients such as color and flavor must be maintained during storage and distribution. Most dairy ingredients contain lactose, which is a reducing sugar that can interact with amino acids and cause non-enzymatic browning in powders. Whole milk powder and dried cream have shelf lives of less than a year because of their high fat concentrations (minimum 26% and 42% fat, respectively), which leads to the development of oxidative rancidity. Nitrogen flushing of packaging containing milk powders has been shown to improve the shelf life of stored powders that are higher in fat (Lloyd, et al., 2009).

Segregation of ingredients in dry mixes during bin storage, bulk transport, and packaging also can be an issue. Ideally, all of the ingredients have the same particle size to prevent segregation, and powders are blended in a ribbon blender to achieve the most homogeneous distribution of the different components. The fill level, blade type, and blade speed of the blender all affect the ability to achieve a homogeneous mix (Muzzio et al., 2008).

Beverage Mixes

In their simplest form, nonfat dry milk (NFDM) and whole-milk powder are used by consumers to make beverages. The next level of value is added by agglomerating or "instantizing" the powder to improve the ability to reconstitute powders. Such products include chocolate milk, hot cocoa,

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Table 17.1. Steps in the reconstitution of dairy powders.

Steps in powder dissolution	Process	Factors affecting
Wetting	Tension of surface is overcome and water is in intimate contact with dry particle	Particle porosity and pore radius Fat content: more fat, more hydrophobicity, less wettability Lactose crystallization causes caking and reduces wettability Age and temperature of storage
Sinking	Particles fall below the surface of the liquid; begins process of dissolution	Higher particle density, better sinkability Lactose crystallization increases sinkability More fat, less lactose crystallization, less sinkability Swelling of particles when wetted reduces sinkability
Dispersing	Agglomerates disperse into additional particles	Large particles increase dispersibility; as the percentage of fines increases, dispersibility decreases Higher porosity and density, more dispersibility
Dissolving	"Soluble" milk components such as lactose, whey proteins, and minerals dissolve and casein is fully dispersed	pH and temperature of the solution Increased storage time increases cross-linked proteins, which decreases solubility Increased denaturation decreases dissolvability

Fang et al. (2008)

nutritional and diet shakes, and smoothies. As with puddings and dessert mixes, many beverages that contain significant amounts of milk powder, whey, or caseinates as part of their formulation rely on the consumer to add liquid dairy products. These products are typically dry blends in which ingredients are blended in a ribbon blender, similarly to cake mix powder blending operations. In some cases, sweetened and flavored and fortified liquid milk or whey (especially mixed with cocoa) are spray-, tray-, or drum-dried.

A number of malted, milk-based beverage mixes that were developed in the early 1900s as nutritional and energy beverages are still popular today. They are fortified with vitamins and minerals and contain a malted ingredient such as malted barley or malt extract as well as milk solids. The dry mix is then added to hot or cold milk or water, depending on preference. Examples of these beverages include Milo (Nestlé), Horlicks (GlaxoSmithKine), and Ovaltine (trademark of Associated British Foods and made by Twinings or Nestlé, depending on the country). Examples of hot and cold beverage mixes are shown in Tables 17.2 and 17.3.

Cocoa beverage powder compositions containing nonfat dry milk (10% to 30% NFDM; 10% to 60% cocoa; 25% to 60% sucrose, salt, and flavorings) are provided by Johnson and Peterson (1974). This powder is added to hot water or milk. They give another recipe without NFDM for use in milk. It consists of 20% to 30% cocoa, 70% to 80% sucrose, and some lecithin, salt and flavorings.

Table 17.2. Instant hot beverage mixes.

Percent Composition	Hot cocoa mixes	Instant coffee beverages
Nonfat dry milk/whey/ buttermilk powder	30–40	10
Sucrose/lactose or other carbohydrate solids source (corn syrup solids, maltodextrin)	30–50	60–80
Cocoa	4.00	0
Instant coffee	0	4
Added fat	0–8	1–5
Stabilizers, emulsifiers, fortification	1–2	1–2

Dry Non-dairy Coffee Whitener/Creamer

Non-dairy creamers are used as substitutes for dairy cream products and to create versatility of new specialty flavors. They are more economical than natural dairy products. Non-

Table 17.3. Instant chocolate milk drink mixes.

Percent Composition	Sweetened breakfast beverage	Sugar-free breakfast beverage	Chocolate milk
Concentrated protein source (milk protein, whey)	13	22	0–20
Sucrose/lactose	50	0	60–70
Cocoa	9	17	10–20
Carbohydrate solids source (corn syrup solids, maltodextrin)	6	22	0
Added fat	9	17	0
Stabilizers, emulsifiers, fortifiers, high-intensity sweeteners, flavors	13	22	10–20

dairy creamers are available in extended-shelf life liquid and shelf-stable dried form. The powders are produced by spray drying the liquid counterpart.

Non-dairy creamers are oil-in-water types of emulsions. The formulation includes an oil (melting point 35°C to 40°C [95 to 104°F]) such as palm oil, palm kernel oil, coconut oil, cottonseed oil, partially hydrogenated soybean oil, canola oil, and sunflower oil. The oils substitute milk fat for cream and deliver the creamy flavor of cream. Maltodextrins and 20 to 24 dextrose-equivalent (DE) corn syrup solids simulate the sweet flavor of the lactose contained in cream as well as mouth feel. Cane sugar is used in some formulations. Stabilizers including carrageenan and sodium caseinate prevent oil separation from the aqueous phase by giving stability to the emulsion. Sodium caseinate contributes the white appearance to the creamer by reflecting light from the casein-coated fat particles. Some low-fat creamers contain titanium dioxide for a whitening effect.

Emulsifiers (e.g., lecithin, polysorbate 60, mono- and di-glycerides, and sodium stearyl lactylate) assist in creating an emulsion with a desirable texture and flavor. In addition, natural and artificial flavors are used to mimic cream flavor. Other flavors impart a variety of gourmet tastes to creamers. Buffering salts such as dipotassium phosphate tend to prevent curdling ("feathering") as it comes in contact with hot coffee, which is naturally acidic in nature.

The manufacturing process for dried non-dairy creamer involves the preparation of a fluid formula, followed by spray drying. Formulations vary widely. Earlier products contained, on a dry basis, 60% to 65% corn syrup solids and maltodextrins, 20% to 32% vegetable oil of 35°C to 40°C (95 to 104°F) melting point, 2% to 5% sodium caseinate, 1% to 3% dipotassium phosphate, and 1% to 3% emulsifiers, stabilizers, and cream flavors (Gardiner, 1977). Another suggested formula is 20% palm oil; 36% corn syrup solids; 1.3% sodium caseinate; 2.8% lecithin, mono-, and diglycerides and stabilizer; 0.3% salt; and 39.6% water. The ingredients are slurried in water at 60°C to 70°C (140 to 158°F) to obtain 65% to 70% solids, and blended thoroughly under vacuum to avoid air incorporation. The blend is homogenized on a two-stage homogenizer and pumped through a spray dryer and then a fluidized dryer to yield an agglomerated powder. The dryer is set at an inlet temperature of 174°C (345°F) and an exit temperature of 114°C (237°F). The product should be free-flowing and dissolve in hot coffee instantly without floating white particles on the coffee surface or sediment on the bottom. Other product quality parameters include creamy flavor along with strong whitening power.

Puddings and Dessert Mixes

Instant and cook-up puddings, pie fillings, and mousses are formulated with milk or cream and something to thicken the product such as gelatin, starch, or another carbohydrate-based gum. Many powdered dessert dry mixes do not contain the dairy ingredients, or

they do not contain all of the dairy ingredients required for making the product, and instead rely on the consumer to add the milk and cream. This makes the dry mix less expensive, and prevents issues common with storing dried dairy ingredients such as caking, browning, and oxidative rancidity.

Crème Brûlée manufactured by Dr. Oetker is an example of a commercial mix that contains significant amounts of dry dairy ingredients including whey powder (fourth ingredient), dried cream, sodium caseinate, and skim milk powder. Milk and cream are added to the dry mix to make the dessert. No-bake cheesecakes such as Jell-o No Bake (Kraft Foods, Glenview, IL) are another example; they contain cheese powder, caseinate, sweeteners, emulsifiers, and stabilizers. Cold milk is whipped with the dry mix, and the blend is set by the starch and gums in the mix.

Dry mixes are intended to have a shelf life of at least one year at ambient conditions. Therefore, when powdered cream, whey, and milk powder are included in these mixes, it is very important to package them in foil-lined pouches, and ideally nitrogen flush them.

Cheese Powders

Cheese powders are used for seasonings and sauces in products such as packaged meals and side dish kits, snack seasonings, dry dip mixes, and soups. They are produced by grinding cheese, diluting it with water, and adding additional ingredients such as flavors, spices, whey, milk powder, vegetable oil, emulsifying salts, highly flavored enzyme modified cheese, carbohydrate-based extenders (maltodextrins, corn syrup solids), anti-caking agents, acids, and color. The mixture is pasteurized and then spray dried.

Quality issues with cheese powders include non-enzymatic browning as well as oxidative rancidity due to the high level of fat in the product (often up to 50%). Cheese itself has lower levels of sugars necessary to participate in non-enzymatic browning reactions, but ingredients added to cheese powders such NFDM and whey contain large amounts of lactose. The water activity and storage temperature of the product affect the browning reaction in cheese powders; therefore, formulation and distribution conditions can be manipulated to minimize browning (Kilic et al., 1997). Packaging is an important consideration in cheese powders. The product is often nitrogen flushed and stored in foil-lined packages to reduce oxidation.

Dairy Ingredients in Dry Mixes

Nonfat dry milk. NFDM is added because it contains 35% protein in the natural proportions of casein and whey proteins, and is relatively resistant to lipid oxidation due to its low fat content (less than 1.5%). Nonfat dry milk has a shelf life of 12 to 18 months if appropriately packaged and stored. Instantized milk powder has a shorter shelf life (about six to 12 months). NFDM is added for whipping air into products, binding water, gelation, and flavor. It is classified by the drying method and the amount of heat applied.

As an ingredient in a dairy product, low-heat, spray-dried NFDM is typically used because it has the cleanest flavor, less protein denaturation, and less carmelization of the lactose. High-heat, spray-dried nonfat dried milk is used for bakery applications, and roller- (drum) dried milk powder is used for milk chocolate because of the extensive carmelization of the sugars that will have occurred.

For spray-dried milks, the American Dairy Products Institute classifies heat treatment as low, medium, and high by measuring the amount of undenatured whey protein in a sample. As heat treatment progresses, whey protein is denatured and becomes less soluble. Additionally, in the United States, milk powders are further classified by the U.S. Department of Agriculture (USDA) as extra

grade or standard grade, or they are not assigned a grade for failure to meet standards. Standards are based on flavor, appearance, bacterial counts, fat and moisture content, solubility, scorched particles, and titratable acidity. Extra grade has a lower maximum milk fat (1.25%); less moisture (4% vs. 5% for standard); and more stringent solubility, titratable acidity, bacterial, and scorched index requirements. There are different solubility and scorched index requirements for spray-dried and roller-dried milks (USDA, 1984; USDA, 2001a,b,c).

Whole milk powder. Whole milk powder contains 26% to 40% fat (or up to 42% fat outside the U.S.). Whole milk powder is added to provide fat and functional milk proteins to a product. It is not classified on the basis of heat treatment received, but it is categorized by the USDA on the basis of flavor, appearance, bacterial counts, moisture content, solubility, scorched particles, and titratable acidity into "extra" and "standard" grades, as with nonfat dry milk (USDA, 2001).

Nitrogen flushing of packages containing milk powders has been shown to improve the shelf life of stored higher fat powders; thus, oxygen barrier plastic-lined bags are often used for packaging and nitrogen flushing (Lloyd et al., 2009). Due to issues with fat oxidation, labeling of the maximum oxygen content as a percentage is often optionally provided on supplier specifications and packaging. Dry whole milk has a shelf life of six to nine months at ambient temperatures, but it can be extended by storing it at refrigerated temperatures.

Dried cream. Dried cream is added to provide fat for functionality and flavor. In the United States it can contain between 40% and 75% fat, according to the allowance in the Code of Federal Regulations for blending dried milk powders or lactose with dried cream to obtain desired fat and protein levels. International regulations such as the Codex Alimentarius Standard 207–1999 require a minimum of 42% fat. Emulsifiers, stabilizers, anti-caking agents, antioxidants, and nutritive sugars may be added.

As with whole milk powder, dried cream can easily oxidize. It also can create issues with processing because it does not flow the same as fat-free powders and can stick to the walls of spray dryers. Therefore, other ingredients are added when manufacturing dried cream. Cream is separated from milk, then ingredients are added to help emulsify and stabilize the fat. These ingredients include caseinates, whey and milk protein concentrates, milk permeate and retentate, and skim milk powder (Havea et al., 2009). Lecithin is often added to help with dispersion of milk fat in the dried product. The liquid blend is then spray or drum dried, and flow-agents can be added to the cooled powder. Oxygen barrier plastic bag liners are used for packaging, and nitrogen flushing is used to reduce fat oxidation.

Buttermilk powder. Buttermilk powder is produced as a waste stream of the butter-making process. When cream is churned into butter, the fat agglomerates and a phase inversion occurs which releases water, protein, lactose, and fat globule membrane material as buttermilk. The U.S. Code of Federal Regulations does not have a standard of identity for buttermilk, other than the labeling requirement that buttermilk can be sweet cream buttermilk, concentrated sweet cream buttermilk, reconstituted sweet cream buttermilk, and dried sweet cream buttermilk. The USDA grades dry buttermilk and dry buttermilk product. Dry buttermilk has a protein content of not less than 30%, whereas dry buttermilk product has a protein content of not greater than 30%. Other than that, the grade requirements for extra and standard grades are identical and both products contain greater than 4.5% milk fat.

Buttermilk powder is unique from skim and whole milk powders in that it contains a higher percentage of phospholipids due to the fat globule membrane material. These

phospholipids provide functional emulsifying properties. The fat globule membrane material also is associated with bioactive attributes from some of its components; Dewettinck et al. (2008) is an excellent review. Because of its fat content, the shelf life of buttermilk powder is less than that of nonfat dry milk, typically six to nine months.

Lactose. Lactose is the disaccharide naturally found in milk. It is commercially produced by removing the lactose from whey when whey is refined to concentrate the proteins by ultrafiltration. The liquid containing the lactose is condensed, and the sugar is allowed to crystallize by gradually cooling the liquid. The crystals can then be further treated by grinding and classifying by granule size (unground, medium, fine) or they can be washed to remove riboflavin, minerals, and remaining protein to create "refined" lactose. It is added to formulas to increase the solids level without greatly increasing sweetness. It also participates in browning reactions through both the Maillard reaction and carmelization. Like other sugars, lactose can help disperse other ingredients such as milk powders and stabilizers, making them easier to reconstitute. Lactose improves dispersibility by physically separating ingredients in a dry blend and adding density to the mix to help with sinkability during reconstitution, or by helping to form complexes during agglomeration processes that improve dispersibility. Chapter 6 of this book deals with dry milk products in detail.

Whey. Whey-based ingredients can take the form of sweet or acid dried whey, whey protein concentrate, whey protein isolates, and modified whey powers. Lower protein, less refined products (sweet or acid whey) are inexpensive sources of solids, whereas whey protein concentrates and isolates are added to foods as protein sources and for their protein functionality. Whey is the waste product from cheese making. Depending on the type of cheese (acid- vs. rennet-coagulated), the whey is either acid or sweet. Acid whey has a much higher mineral content than sweet whey. Both dry powders are approximately 12% protein and 75% lactose, which limits their use for functionality. To further refine whey, it undergoes ultrafiltration to remove lactose and minerals. Whey protein concentrates are available in various ranges of protein, usually 35% protein (to replace skim milk powder) and 80% protein. Whey protein isolates have greater than 90% protein. Modified whey powders have had minerals and lactose removed by various processes such as ultrafiltration or electrodialysis, and are not necessarily sourced from cheese whey. Chapter 8 discusses whey products in detail.

Caseinates. Caseinates are produced by isolating the casein proteins in milk by methods such as acid or rennet precipitation, or filtration, and then often treating to modify protein solubility and functionality (typically creating sodium or calcium caseinate). Caseinates contain greater than 90% protein. They are used extensively in coffee whiteners. Because they also are extremely functional emulsifiers and foam formers, they are very useful in mousse powder blends and powdered coffee mixes where milk foam is desired. See Chapter 7 for a more detailed discussion on caseinates.

Processing of Dry Mixes

Chapters 6, 7, and 8 in this book cover production of dry dairy ingredients; refer to those chapters for more information on drying ingredients and agglomeration processes. The considerations that are unique to powdered mixes are presented below.

Blending

The ideal way to create a powdered mix is to mix, blend or reconstitute ingredients in water or milk, and then pasteurize, homogenize, and spray dry the blend. Further treatments such as particle size enlargement

(agglomeration) can be applied to make the product more easily dispersible in water. Powdered mixes can be created by blending dry ingredients together. Although this method requires much less capital investment and maintenance, it is difficult to incorporate fats and oils, no final pasteurization is possible, there is no ability to agglomerate, and the different powder densities can lead to segregation of ingredients (Montagne et al., 2009). Even in mixes that are blended and spray dried, dry blending often occurs to mix in emulsifiers such as lecithin, and vitamin and mineral premixes. A ribbon blender is used to achieve the most homogeneous distribution of the dry components. The fill level, blade type, and blade speed all affect the ability of the mixer to achieve a homogeneous mix (Muzzio et al., 2008).

Storage and Conveying

Once mixes are completed, it is very important that the powders are handled properly to minimize the reduction in particle size (creation of fines) and the segregation of ingredients based on variations in particle size. Fines can be caused by abrasion of the agglomerates due to the particles rubbing against each other or the equipment. Segregation is due to variations in particle size and density, storage bin design, and air transfers, which can "classify" the ingredients by their size. Segregation problems can be minimized if the ingredient size ratios are not greater than 3 : 1 (Barbosa-Cánovas et al., 2005).

Packaging

Packaging for powders is designed to minimize oxygen and moisture transfer to the product, and the design depends on the sensitivity of the product. The fat content, delicacy of the flavor, and the inclusion of sensitive vitamins are usually the main considerations. Oxidation of the fat in powdered products is a chemical reaction that leads to rancid off flavors that are described as cardboard, painty, and fishy. The oxidation reactions that occur also oxidize sensitive vitamins such as vitamins C and A and riboflavin, causing them to lose their biological activity. Flavoring ingredients also can be destroyed.

Protecting the powder from light, reducing oxygen, removing minerals that initiate oxidation, and adding antioxidants improve the shelf life of powders. Reducing the oxygen in the headspace by flushing with nitrogen is a common way to improve shelf life. Flushing accomplishes nothing, however, if the package does not have good oxygen barrier properties or if the seal is not complete. Metal cans exclude oxygen and moisture quite well. Flexible films based on metallized polyester/low density polyethylene laminates, and metalized paperboard or paper packages also minimize oxygen transmission (Brown and Williams, 2003). Moisture transfer that leads to rehydration of the powder can cause caking and effects easy reconstitution of powders. Humidity fluctuations can cause particles to cake, or stick together. Lactose in milk powder commonly cakes because it is can absorb moisture, cause powders to stick together, and then recrystallize (Barbosa-Cárnovas, 2005). Anti-caking agents can be added to prevent the bridging between particles, as can moisture barrier packages. Metal cans provide an effective barrier, as do films containing polypropylene and ethylene vinyl alcohol (EvOH) (Risch, 2009).

Packaging system also can directly increase or decrease flavors and off-flavors in the product. Flavors can migrate from the package to the product, which is generally undesirable. Plasticizers and solvents from the package can migrate into the product and affect the flavor and safety. Styrene, benzene, PCBs, and, most recently, bisphenols have been found to migrate into food from packages, and in the case of styrene, can

cause detectible flavors (Brown and Williams, 2003). Solvents and plasticizers used in inks for shrink labels that cover packages also can migrate through plastic packages into the food (Bradley et al., 2005). Packaging material can also scalp desirable flavors and lead to bland products; therefore, it may be necessary to evaluate different package materials to maintain the desired quality in a product. Packages can protect the product from exterior odorants in the environment surrounding the package. Thus, a barrier layer in the package, as with moisture and oxygen protection, can improve flavor transfer and retention.

Another consideration is the effect of package size on the product consistency. When there is a range of particle sizes in the powder, packaging products in individual serving packages instead of bulk canisters reduces the probability of consumer disappointment with inconsistent products caused by classification of the powders within the package during distribution.

Frozen Desserts

A wide variety of ingredients are used in frozen desserts. For standardized products in the United States, the allowable ingredients are defined by the Food and Drug Administration (FDA) in the Code of Federal Regulations (CFR), section 135 (CFR, 2010). The frozen products standardized in the CFR that contain milk products include ice cream, frozen custard, goat's milk ice cream, sherbet, and mellorine. Most frozen dessert mixes are formulated, processed, and extruded through ice cream freezers to deliver desirable consumer attributes of flavor, texture provided by fat and emulsification of ingredients, and stability of the products.

Ice Cream and Related Products

The main components of frozen dairy products are air, water, milk fat, milk-solids-not-fat (MSNF), sweeteners, stabilizers, emulsifiers, flavors, particulates, variegates, and coloring materials that are typically mixed in after freezing. Products are frozen by a number of techniques such as continuous churn ice cream freezers, batch freezers, and soft-serve machines, all of which are discussed later in this section. The machines freeze the water portion of the mix and harden the fat while air is whipped into the base to reduce its density. The reduction in the density of the original base is termed overrun. A base that weighs 9 lbs/gallon before processing and 4.5 lbs/gallon as finished frozen dessert has 100% overrun. Ice cream in the United States must contain at least 10% milk fat and at least 10% milk MSNF (milk protein, lactose, and minerals). As the fat content of ice cream increases, there is an allowable decrease in the MSNF due to dilution (Table 17.4).

For ice creams without bulky flavors, the weight of milk fat cannot be less than 10%, and the total milk solids cannot be less than 20%. There is also a provision for ice cream to weigh at least 4.5 lbs/gallon to prevent economic fraud by excessive incorporation of air. Ice cream can contain egg yolk solids, but they must be present at less than 1.5% by weight to be called ice cream. Frozen custard (also called French ice cream or French custard ice cream) meets the same requirements for fat and milk solids as ice cream, but the product must contain at least 1.5% egg yolk solids. Provisions are made for the addition of bulky flavors to both products to

Table 17.4. Minimum solids required in ice cream in the U.S. CFR as milk fat increases above 10%.

Percent milk fat	Minimum percent nonfat milk solids
10	10
11	9
12	8
13	7
14	6

Adapted from CFR (2010)

Table 17.5. Typical formulation of various grades of hard-frozen ice cream.

	% Composition		
	Economy ice cream	Regular	Deluxe or super-premium
Butterfat	10	12	16
Milk-solids-not-fat	11	10.00	8
Sugar	9.5	12.00	15
Corn syrup solids	7.8	4.11	0
Stabilizer/emulsifier	0.3	0.25	0.00
Egg yolk			variable
Total solids	38.6	38.25	39
Weight/gal	45 lb	45 lb	5.5–6 lb

Tressler (1975), www.foodsci.uoguelph.ca/dairyedu/icform.html 2010

allow for the reduction in milk fat and solids and egg solids. The composition of ice cream products varies depending on the value proposition required by the manufacturer. For premium ice cream, higher fat and higher quality ingredients are used. Less fat, more air, and less expensive dairy ingredient are used in value brands. See Table 17.5 for a comparison of ice cream types.

The European Union standards for ice cream are much less restrictive and can include a product comprised of water and/or milk, edible fats (from non-dairy sources), proteins, and sugars, with no minimum quantity requirements. This is similar to the definition for the standardized product mellorine in the United States (CFR, 2010), except that mellorine has minimum fat and solids requirements. Gelato is included in the European definition for ice cream. Milk ice and dairy ice cream are more equivalent to American ice cream: all of the fat and protein must be exclusively from dairy sources; however, egg is allowed. For milk ice, the minimum requirements for fat and MSNF are 2.5% and 6%, respectively. "Dairy ice Cream" must be at least 5% milk fat (European Ice Cream Association, 2006). Crema gelato is included in this definition.

Low-fat, fat-free, and sugar-free frozen dairy products have been developed over the last couple of decades, and the sensory properties of these products have greatly improved by the development of novel fat-replacing ingredients and industrial know-how. A survey of nonfat ice creams shows that combinations of cellulose, guar and carob bean gums, carrageen, and polydextrose are commonly used in fat-free products to provide body and mouth feel.

Soft-serve ice cream and frozen yogurt are served immediately after being frozen, with no hardening. Milkshakes are similarly formulated. These products are generally lower in fat than hard-pack ice cream, and carbohydrate- and protein-based fat substitutes are used extensively to provide body and texture to the lower-solids product. The fat provides a creamy texture, although the possibility of having a coarse or icy texture increases if texture modifiers are not used. The MSNF content varies from 10% to 16% and the total solids content varies from 30% to 35%. Compared with hard ice cream, soft frozen desserts contain higher serum solids, lower sweetener levels, and lower overruns (30% to 60%). Frozen yogurt has no standard of identity for the amount of yogurt, but typically skim milk is incubated with yogurt cultures to produce acid, and when the correct pH is reached, the yogurt is cooled and then combined with a pasteurized sweetened base that includes the stabilizers and the balance of the soft-serve mix ingredients. Table 17.6 compares the composition between several soft-serve products.

Table 17.6. Comparison of soft-serve frozen yogurt, ice milk, ice cream, and milkshake base.

	% Composition			
	Frozen yogurt	Ice milk	Ice cream	Milkshake base
Butterfat	2	4	10	4
Milk-solids-not-fat	14	13	12	12.5
Sugar	15	12	11	13
Corn syrup solids		4.5	3	5
Stabilizer	0.35	0.5	0.4	0.4
Total solids	31.35	34	37.4	34.9

Adapted from Tressler (1975), www.foodsci.uoguelph.ca/dairyedu/icform.html 2010

Table 17.7. Typical composition of milkshake.

	% Composition			
	Direct draw shake*		Blended flavored shake**	
	Smooth and thick	Coarse & icy	Smooth and thick	Coarse & icy
Butterfat	0–4	0–4	0–4	0–4
Milk-solids-not-fat	12	10	12.5	115
Sugar (Equivalent to sucrose)	12	12.5	9	8
Stabilizer/emulsifier	0.5	0.2	0.35	0.2
Total Solids	24.5	22.7	21.85	19.7

* Draw temperature: –5°C to –4°C (23°F to 24°F), 50% overrun
** 1 to 2 oz syrup added at spindle stage contributes 5% to 7% sweetener (sucrose equivalent) in 16-oz shake. Draw temperature: 22°F to 23°F (–5.6°C to 5°C)
Adapted from Chandan (1997)

Milkshakes can be formulated to give certain consumer attributes that vary in their texture and mouth feel. The compositions of two types of shakes are shown below in Table 17.7.

Sherbet is a frozen-milk, sweetened dessert that is often flavored with fruit juice. It is a standardized product in the United States. It must weigh more than 6 lbs/gallon of finished product, have a fat content between 1% and 2%, and a nonfat milk solids content of at least 1%. The total milk or milk-derived solids must be between 2% and 5% by weight of the finished food. Sherbet that is characterized by a fruit ingredient must have a titratable acidity, calculated as lactic acid, of not less than 0.35% (CFR, 2010). To achieve these ranges the ingredients in sherbet are typically water and sugar, followed by dairy ingredients such as milk and cream, and then fruit puree and stabilizers (Table 17.8). Because of the low fat and dairy

Table 17.8. Typical composition of sherbet.

Percent Composition	Non-fat sherbet	Sherbet
Butterfat	0	2
Milk-solids-not-fat	1	2.8
Sugar	16	16
Corn syrup	10	12
Stabilizer	3–5	3–5
Fruit juice, acid, water	To make up remainder (depends on milk solids and fat source)	

solids, sherbet has a coarser, icier texture than ice cream. Like soft-serve ice cream, it relies on carbohydrate-based gums for a smooth mouth feel.

Frozen novelties are a highly variable segment of the dessert market that include products in bar form on sticks, chocolate-covered bars, ice cream between wafers or cookies and ice cream in cones. Particularly important characteristics are the ability to form the product into the appropriate shape; apply the coating, cookie, or wafer and have it adhere; and control moisture transfer to or from the ice cream to the other components. The ice cream or other frozen dairy base in novelties is generally highly stabilized with gums. Novelties are portion controlled because they are usually individually packaged within a box to provide stability to the product. A recent trend is to provide 100-calorie products and low-fat or nonfat or sugar-free novelty varieties. The 100-calorie products can be achieved by reducing the serving size. For reduced-fat and reduced-sugar products, because novelties are a multiple-component system, sensory differences from full fat or full sugar counterparts can be partially masked with the other components so that satisfactory sensory characteristics can be experienced.

Purpose of Dairy Ingredients in Frozen Desserts

Fat

Fat contributes to flavor in frozen products, but more importantly, it contributes to texture, foam stability, and melt-down in the mouth. Prior to freezing, ice cream mix is blended, pasteurized, and homogenized. The heat during pasteurization fully melts crystalline fat, and homogenization creates smaller fat globules which allow protein and emulsifiers to form new surface interactions with the greatly increased fat surface area. The next step of dessert mix handling is cooling to 4°C (39°F) and holding (also called aging or ripening) the base. This allows for a portion of the fat to crystallize and for reorganization of emulsifiers at the fat interface (Eisner, Wildmoser et al., 2005). During freezing, the mix is aerated while water is frozen and scraped off the sides of the heat exchanger by the dasher. A portion of the liquid milk fat is also crystallized. The resulting product is a mixture of air bubbles with agglomerated fat on portions of the air bubble surface. The source of fat in a frozen dessert can affect its sensory properties. Milk fat contains a large variation in fatty acids, so there is a large melting range and a combination of liquid and crystalline fat during freezing, which contributes to its unique structure development (Goff, 2009).

Sources of Fat

Fresh sweet cream and fresh milk. Whole milk may be used primarily as a source of serum. There is no doubt that sweet cream is the best source of fat for ice cream because of its desirable flavor, convenience of handling, and good whipping characteristics. Fresh cream is judged by flavor, titratable acidity, and microbial count. The developed acidity should be low, and the cream should have been carefully handled prior to pasteurization to prevent the development of hydrolytic rancidity, which is due to free fatty acids from the partial hydrolysis of butterfat. Proper heat treatment, an essential phase for the preparation of cream for freezing, consists of heating cream at 76.7°C (170°F) for 20 minutes, or at 82.2°C (180°F) for 10 minutes, or at 87.8°C (190°F) for 5 minutes. This treatment not only inactivates the lipase enzyme naturally present in milk, but also destroys 95% to 99% of the bacteria present.

Frozen cream. Cream is commonly stored in a frozen state to offset its short shelf life and the high price of sweet cream during certain seasons. When storing frozen cream

it is necessary to prevent off flavors from developing. Only the best cream should be processed for storage, and it should contain no developed acidity. Off flavors that are likely to develop in frozen cream are oxidized, rancid, fishy, oily, and tallowy. A fishy flavor in dairy products occurs when trimethylamines are formed by the hydrolysis and oxidation of lecithin, a naturally occurring phospholipid in milk. Factors which promote development of this flavor are high acidity and the presence of pro-oxidants such as iron or copper salts. Heat-treating cream helps it to resist oxidation (Ewbank and Gould, 1943). Following heat processing, cream intended for frozen storage should be frozen quickly. Proper packaging and handling of frozen cream is also important; stainless steel or plastic containers are preferred. Quick-frozen cream is held at −23.4°C (−10°F) or lower. Disadvantage of frozen cream are the necessity of thawing it prior to use, and handling issues.

Butter. Fresh sweet-cream unsalted butter is often used for ice cream, as it is often the least expensive source of fat. Sweet cream butter has been found to whip more slowly due to loss of natural emulsifiers (the phospholipids) in the buttermilk fraction. However, this is easily overcome with commercial emulsifiers and homogenization. Butter can be stored frozen for an extended shelf life; however, it can suffer the same oxidative defects as frozen cream and it can absorb odors from the environment. Storage in proper packaging is crucial.

Plastic cream. Separating sweet cream (28% to 35% fat) a second time in a special separator bowl at relatively high temperature 60°C (140°F) provides cream with a fat content of 79% to 81%. Because of this high fat content, the product solidifies to a plastic or solid mass upon cooling, and is therefore known as plastic cream. If the product is cooled quickly to 4.4°C (40°F), it remains in a liquid state which allows for immediate packaging because crystallization of the fat is fairly slow. It can be frozen if it is not to be used immediately.

Butter oil and anhydrous milk fat are rarely used as fat sources in frozen desserts in the United States, but they are popular choices in other parts of the world. Dry cream and dry whole milk also are not widely used in the formulation of ice cream mix because they are prone to oxidative flavor issues.

Milk-Solids-Not-Fat

Milk-solids-not-fat include milk protein, sugar (lactose), and minerals. Depending on the ingredient, the ratio of components varies greatly, which can affect the frozen product's flavor and texture. Fluid whole and skim milk are excellent sources of solids and fat because of their clean flavor. The flavor and texture can be adversely affected as milk products are further processed by condensing, drying, and separation processes (see below). The lactose and mineral portions of the milk solids affect the freezing point of the continuous phase, just as added sweeteners do. Lactose in ice cream can crystallize, causing a flavor defect known as sandiness. This can occur at high concentrations of milk solids or when adding ingredients with high concentrations of lactose such as acid or sweet whey. Fluctuations in the storage temperature of ice cream also contribute to the formation of lactose crystals. The proteins contributed by the milk solids help with emulsification, aeration, and foaming, and they contribute to the stabilization of air bubbles, and water holding (which contributes to viscosity in the continuous phase).

Sources of Milk-Solids-Not-Fat

Fluid whole and skim milk. Both fluid whole and skim milk are excellent sources of MSNF and should be used in the mix whenever they are available at a reasonable cost.

However, because of their low serum solids content in contrast to the serum solids desired in an ice cream mix, their use is limited.

Plain condensed skim milk. Plain condensed skim milk is the source of serum solids that is used more frequently than any other of the condensed products. Fresh condensed skim milk is easy and convenient to use, has an excellent flavor, and is readily available. The composition varies from 25% to 40% total or serum solids, depending on the condensing operation and the distance the product will be transported (higher solids contents are less stable to transportation). The concentrate is purchased on a basis of the solids content. The heat treatment given the fluid skim milk is usually the same as the regular pasteurizing range. The keeping quality of condensed skim milk is better than that of cream. It should be stored at 0° to 1.7°C (32° to 35°F) and used while fresh and sweet (usually seven to 10 days).

Plain condensed whole milk. Plain condensed whole milk is concentrated approximately two and a half times and contains about 8% fat and 20% serum solids. Condensed whole milk is frequently used in ice cream because it is a convenient source of both serum solids and fat. Superheated condensed skim or whole milk is made by slowly heating the already condensed product to a high temperature, usually in the range of 82.2°C (180°F). When done properly, concentrate has a much greater viscosity. When used in the mix, it improves the whipping ability and contributes a smooth texture. Superheated condensed milk permits ice cream manufacturing without stabilizers, and may offer a marketing advantage for certain consumers. Superheating increases the hydration capacity of the milk proteins, which bind more free water. Accordingly, less water is available to form ice crystals during freezing and shelf life. The ice cream's smooth texture is maintained during its shelf life; thus, superheating functions like a stabilizer.

Frozen condensed whole or skim milk. Freezing condensed whole or skim milk is a fairly expensive way to store solids; as a result, they generally are not used. Evaporated canned milk also is not generally used due to the mandatory sterilization heat treatment required in its production, which imparts a noticeable cooked flavor and carmelized color. Sweetened condensed whole milk or skim milk are sometimes used as a source of MSNF, providing 8.5% fat and 28% total milk solids. The added sugar (40% to 44%) improves the keeping quality over that of plain condensed milk. The sugar concentration makes the osmotic pressure of the solution high enough to suppress the growth of practically all microorganisms.

The titratable acidity test should be applied to all condensed milk products. When diluted to contain the same MSNF concentration as skim milk, the titratable acidity should be approximately that of fresh skim milk (0.18%). Sweetened condensed products have a cost disadvantage and are not very popular in the ice cream industry. The freight cost of shipping sugar in the form of sweetened condensed milk is more than that of dry bagged sugar.

Dry buttermilk and condensed buttermilk. Sweet cream buttermilk is obtained from churning cream that has not developed noticeable acidity. Buttermilk has beneficial effects on the whipping ability of the mix due to the inherent lecithin content. It also contributes to a richness of flavor. Dried buttermilk lipids tend to deteriorate readily in storage, so the typical shelf life is six to nine months. Care must be taken to use only fresh ingredients. The fat content of condensed dry buttermilk is 3% to 4% and should be taken into account when calculating the ice cream mix.

Nonfat dry milk solids (NFDM). NFDM is one of the most concentrated sources of serum solids that is frequently used. Spray-dried powder should have good flavor and

light color, and be free from scorched particles and easily dispersible. NFDM should be bought only in such quantity as can be used before the product develops off flavors and kept in cold storage. This slows the development of a stale flavor that can impart an old or storage flavor defect to ice cream. It can be stored up to one year without loss in quality. For use in ice cream mix, extra-grade low- or medium-heat NFDM is recommended. There is no great advantage in using more expensive agglomerated or instant NFDM for frozen desserts. Incorporation of dry milk into ice cream mix is facilitated by the use of a powder funnel and blender pump or special blending equipment.

Dry sweet whey. Dry sweet whey is commonly used at a 25% replacement level of serum solids in ice cream. Because it contains 72% lactose as compared to 52% lactose in nonfat dry milk, its incorporation beyond the suggested level may result in crystallized lactose, the cause of the sandiness defect in ice cream. Delactosed whey, demineralized whey, and whey protein concentrates are not frequently used due to cost constraints, but they have been investigated for their texture-modifying properties.

Sodium and calcium caseinates. Sodium and calcium caseinates improve the whipping properties of the mix, heat-shock resistance, body, and texture of ice cream. Sodium caseinate at about the 0.5% level in the mix accords a slow-melt character to ice cream. However, these ingredients are relatively expensive and used very rarely.

Hydrolyzed milk proteins. Hydrolyzed milk proteins may be derived from casein or whey fractions of milk. They act as functional stabilizers in frozen desserts and can replace gelatin, gums, celluloses, alginates, and other hydrocolloids. Although they are expensive ingredients, they may be preferred for their consumer-friendly appearance on the label. Hydrolyzed whey proteins are subject to a limitation of 25% serum solids replacement.

Reduced lactose products. To improve heat shock resistance and the sandiness defect of frozen desserts, reduced lactose concentrated milk can be used as a source of serum solids. The lactose-reduced ingredient is manufactured by holding the product at room temperature to effect lactose crystallization (subsequent to the condensing step in a vacuum pan). Crystallized lactose is centrifuged out to obtain the reduced lactose product. It has been used in high-solids ice cream mixes. Approximately 25% of serum solids can be replaced with low-lactose skim milk. Another approach for lactose reduction is to use food-grade lactase to effect hydrolysis of lactose prior to the condensing step.

In some cases, ultrafiltration of skim milk to obtain retentate of reduced lactose content has been employed. Removal of lactose from skim milk increases the protein content, which in turn increases the acidity and viscosity of the mix. However, the texture and storage quality of the frozen dessert is markedly improved. Ice cream mix containing lactose-hydrolyzed serum solids freezes at a lower temperature and does not become too hard in storage. Because of conversion of disaccharide lactose to the monosaccharides glucose and galactose, a noticeable depression in freezing point is affected. Accordingly, at draw and storage temperature, the ice cream displays a noticeably softer texture which is an interesting consumer feature. Lactose hydrolysis, therefore, provides a tool to favorably alter the melting properties of ice cream. Use of reduced lactose serum solids, on the other hand, leads to no difference in melting characteristics of the frozen dessert.

Lactose hydrolysis also affects the sweetener level. Because lactose is only 16% as sweet as sucrose, lactose hydrolysis products are much sweeter (53% as sweet as sucrose). Thus, the sweetener content of the mix can be reduced a little when lactose hydrolyzed skim milk is used. From the standpoint of formulation, various grades (or lines) of ice

cream are obtained by changes in the type and concentration of the ingredients.

Processing of Frozen Desserts

Mix Preparation

In all frozen desserts, processing starts with standardization of the base and pasteurization and homogenization. Dairy ingredients are combined to provide the correct solids and fat contents. This may occur as a mix of liquid and dry ingredients. Typically, the liquid dairy ingredients, water, and liquid sweeteners are combined and the mix might be checked for solids and fat. The dry ingredients such as dry dairy powders, sugars, stabilizers, emulsifiers, and cocoa powder are added to the liquids through a blender, either in-line through a tri-blender, or through recirculation through an agitated tank. In some cases, a pre-blend of dry ingredients is made with sugar to ensure that the stabilizers can be incorporated without forming "fish eyes," which can clog in-line strainers. Some ingredients that are difficult to solubilize may need to be pre-heated in water and added as a slurry to the mix.

Because of the amount of stabilizer in the mix, it is important to not incorporate too much air during the blending stage and causing foam. Problems are encountered with pasteurization by continuous pasteurization systems if there is excess foam because the foam may not be able to be pumped, there is burn-on in the plates, and tank volume meters are affected by the less-dense foam. Bases are usually processed as a "white base," which is simply sweetened and ready for flavor addition post-pasteurization, and a "chocolate base," which has had the cocoa powder added but no flavors. When products are pasteurized in small batches through a batch pasteurizer, or when large volumes of a dry ingredient system include the flavor (such as eggnog), the flavors might be added during blending and prior to pasteurization.

Pasteurization

The main purpose of pasteurization is to ensure the safety of the product and destroy any pathogenic organisms. Because of the high solids levels, the temperature requirements to achieve safe products are higher than those for fluid milk (Figure 17.1). The heat treatment at this step also contributes a cooked flavor to the mix, increases the viscosity by denaturing proteins, and incorporates ingredients and affects their functionality (especially emulsifiers and stabilizers). The heat also melts the milk fat so that it is in a liquid state when it is homogenized, resulting in homogenization efficiency.

Homogenization

Homogenization of the mix occurs immediately after pasteurization while the base is still hot in the case of batch pasteurization, or after pre-heating the base during continuous pasteurization. Homogenization completes the mixing of ingredients, reduces fat globule size, further denatures proteins to improve water binding, and aids in the formation of new "membrane" material surrounding the fat (comprised of proteins and added emulsifiers). In milk, the reduction in fat globule size is intended to prevent the fat from rising to the top of the liquid. In addition, if the mix is not homogenized, the ice cream can have a churned defect, evidenced by greasy butter on the blades of the freezer and greasiness of the ice cream.

When homogenized on a two-stage homogenizer, 17.2 MPa (2,500 psi) is typical (14 MPa [2,000 psi] first-stage, 3.5 MPa [500 psi] second stage) for mixes under 14% fat. Mixes with a fat content over 14% have lower homogenization pressures to prevent excessive mix viscosity (Marshall et al., 2003). Products intended for soft-serve applications are packaged at this point. Frozen yogurt manufacturing also has the addition of culture prior to packaging or proceeding to

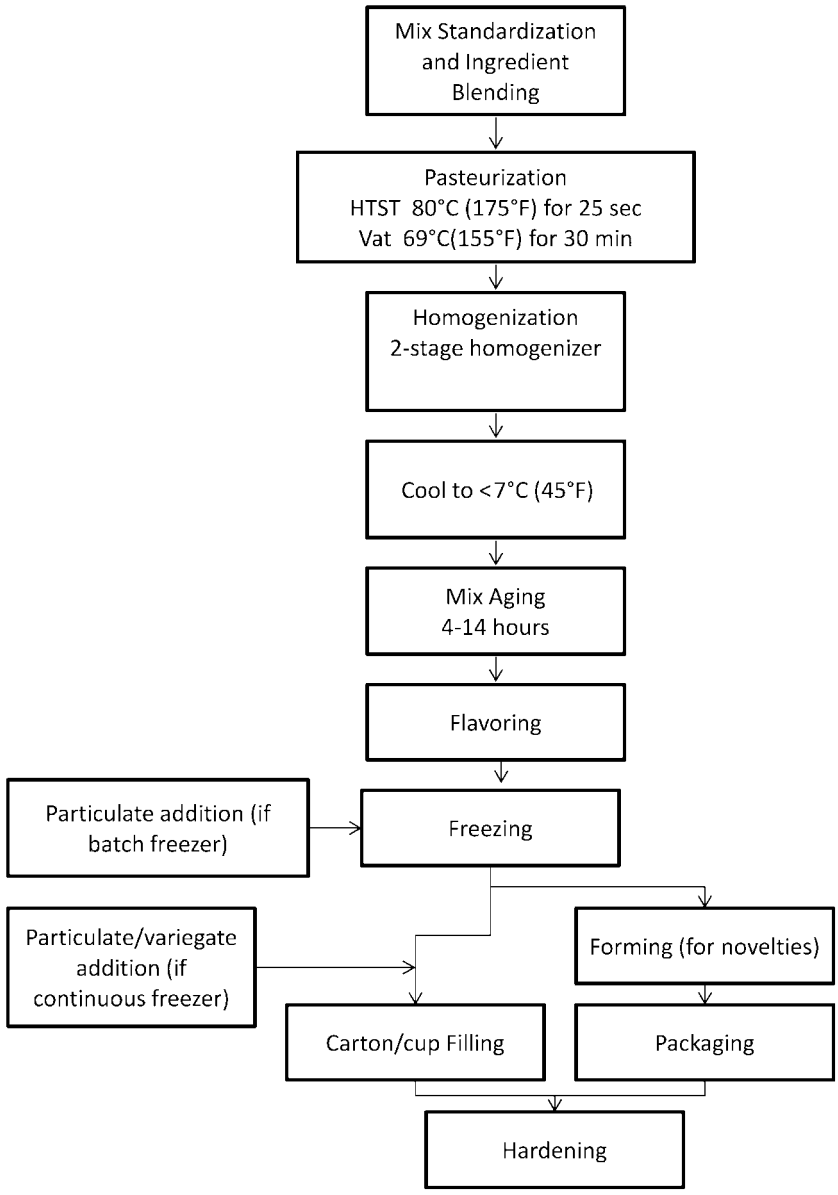

Figure 17.1. Frozen dessert manufacturing outline.

aging and freezing. Frozen yogurt manufacture is illustrated in Figure 17.2.

Aging

Aging is the holding of the cooled ice cream base for a period of time to allow for full hydration of all ingredients, crystallization of some of the milk fat, and time for emulsifiers and proteins to associate with the fat globule and interact at the interface. Traditionally, this has been an overnight process with the base being held for up to 20 hours, depending on the stabilizers that are used. With modern

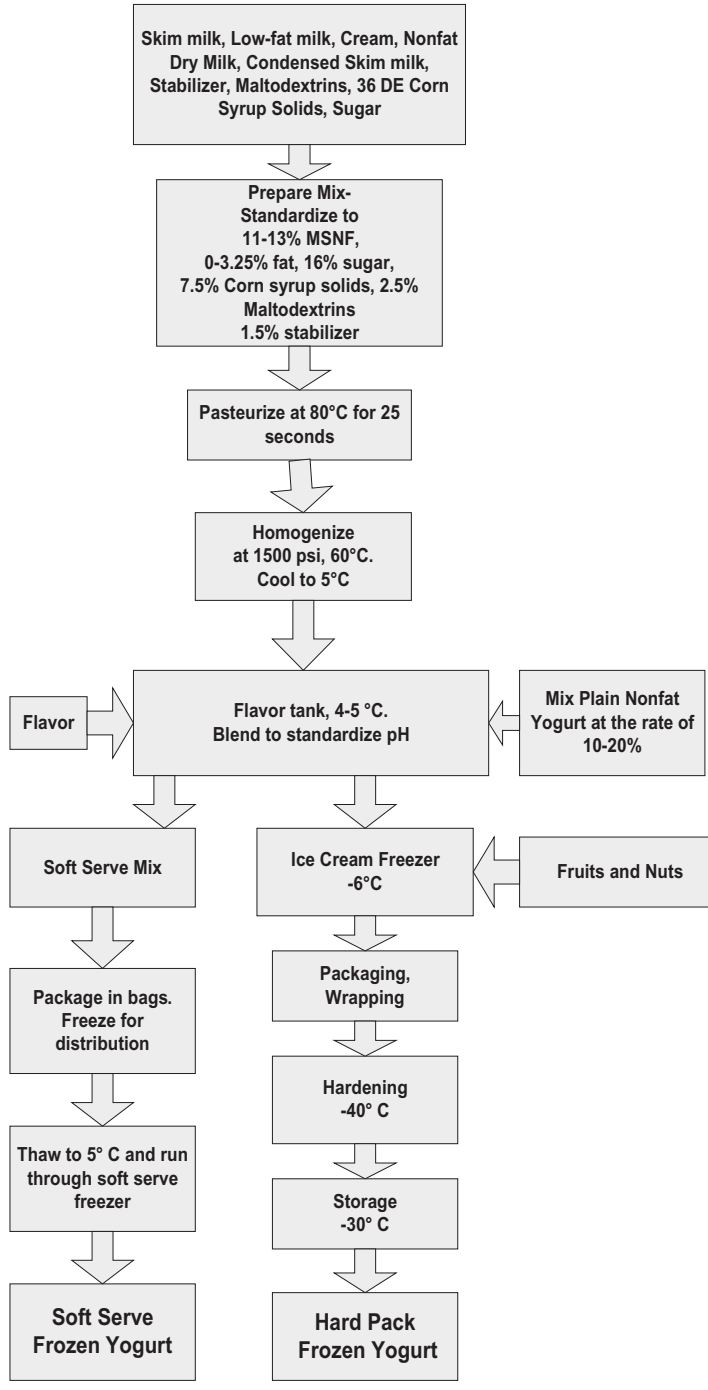

Figure 17.2. An outline for the manufacture of soft and hard frozen yogurt.

stabilizer systems, base is typically held for a minimum of four hours. Bases frozen without aging show evidence of improper emulsification, which is observed by the rate of meltdown of the finished product and churned fat in the freezer (Marshall et al., 2003).

Flavoring

Base is flavored immediately before freezing if the flavors are not added to the mix before pasteurization. This can be done in agitated batch tanks, or injected in-line using a pump and variegate nozzle, with a metering system (mass-flow or mag meters, or loss in weight of the flavor on a scale) to inject the proper amount and a static mixer to thoroughly blend the flavor. In the case of batch freezers, additional bulky ingredients or variegates can be added to the freezer itself and folded in just prior to discharge from the freezer. In the case of continuous freezers, bulky ingredients cannot be added to the freezer and are added after the ice cream exits the freezer by means of a fruit feeder, which mixes nuts, cookies, and other pieces into the base with a dynamic mixer. Swirls and variegates are added in-line by using variegating nozzles to inject syrups into the flowing ice cream.

Freezing

During freezing, aeration of the mix results in a suspension of crystallized fat and water in a concentrated sugar solution that also contains emulsifiers, proteins, and stabilizers. The structure of the fat crystals contributes to the foam stability, texture, and melt-down properties (Goff, 2009). Two types of ice cream freezers are used in commercial production, batch and continuous. Both are scraped surface heat exchangers: they employ a barrel design in which water in the mix is frozen on the surface of the barrel by means of refrigerant in the barrel walls, and then scraped off the surface with a bladed dasher. Air is whipped in by the rotation of the dasher. The main differences between the two designs are the surface-area-to-mix-volume-ratio (continuous barrels have the greater surface-to-volume ratio, hence faster freezing), the dasher tolerance for inclusions, and the amount of overrun that can be whipped into the product (continuous freezers can achieve higher overrun). Batch freezers typically freeze the base within eight to 10 minutes, whereas with continuous freezers the base residence time is measured in seconds; however, this depends on the freezer design and desired overrun.

The important characteristics of the freezing process are the creation of small ice crystals (less than 30 μm), the crystallization of liquid milk fat and aggregation of fat clumps to help stabilize air cells, and incorporation of air to create overrun (Marshall et al., 2003). An additional process that has been developed after continuous freezing allows for improvements to the texture of reduced fat products. Low-temperature extrusion (−15°C; −11°F) after classic ice cream freezing (−5°C to −6°C; 21°F to 23°F) greatly increases the shear forces applied to the base. This shearing occurs when a large fraction of the fat has already crystallized, and results in a destabilization of the fat which creates smaller fat aggregates, smaller air cells, and smaller ice crystals (Bollinger, Kornbrust et al., 2000; Eisner, Wildmoser et al., 2005). These changes positively affect the ability to scoop cold ice cream (making it easier) and reduce the melt-down of the ice cream, increasing the perception of creaminess (Eisner, Wildmoser et al. 2005). This process is being used in reduced fat ice-cream to create product with less fat but with the desirable creamy mouth feel.

The consistency of the frozen dessert as it exits the freezer is referred to as either wet or dry (or stiff). The ice content, mix viscosity, size of the air cells, and amount of air all

effect stiffness (Marshall et al., 2003). These factors are controlled by the base formula and freezer design and running conditions. Stiffer products are more desirable for novelties, whereas a wetter ice cream is desirable for filling containers to prevent voids (Kilara and Chandan, 2007).

Packaging

Frozen dessert packages must protect the product from damage during distribution and from dehydration at the surface, as well as provide the proper shape. Package materials vary depending on the product and can range from 100% plastic to plastic-coated paperboard. Product in plastic film usually is sold in paperboard boxes to afford physical protection.

Hardening

Approximately 30% to 40% of the water is frozen after conventional freezing; this ice is present in the sugar-saturated liquid interface (Eisner, Wildmoser et al., 2005). The purpose of hardening is to freeze more of the water in the packaged dessert by reducing the temperature of the product to below −18°C (0°F). As more water freezes, it is incorporated into existing crystals, causing them to grow (Eisner, Wildmoser et al., 2005). Because larger ice crystals cause a coarse mouth feel, it is desirable to freeze the product quickly to minimize ice crystal growth. For novelties, it is crucial to harden the product quickly to prevent deformation during further handling operations.

Fluid Dairy Products: Refrigerated and Shelf-stable

Cream and Whipping Cream

High-fat fluids are mainly used as creamers for coffees and in the formulation of refrigerated desserts and frozen desserts. The fat provides flavor, lubricity in the mouth, and the ability to whip air into products. The higher the fat, the more overrun that can be whipped into the cream without the use of emulsifiers. The state of the fat in the cream, in regard to crystallization and having intact fat globules with intact membranes, are important to achieve maximum overrun. Cream and whipping cream are not homogenized when they are processed because that can cause defects during whipping and agglomeration of crystallized fat during storage and transportation.

To prevent separation of cream products during distribution, carageenan is commonly added at low levels to increase the viscosity of the blend. It also helps improve whipability and stability of the whipped cream. Emulsifiers such as mono- and diglycerides also are added to improve whipability. Sugars may be added to improve the flavor; they also help to increase the viscosity of the liquid phase, which improves the whipped cream stability.

The stability of the protein in the cream to the heat and conditions in coffee is important for all cream products. Flocculation of protein is highly undesirable. The stability of the cream to the temperature, acidity, and minerals (especially calcium) in the coffee water can be improved by using chelating agents and buffers such as sodium polyphosphates. A comparison of fat levels in various cream products by U.S. regulations is presented in Table 17.9.

Half and Half

It has been estimated that 63% of coffee drinkers add a sweetener and/or creamer. The objective is to develop whiter color and moderate coffee flavor (Decker 1999). Half and half (10.5% fat) is a popular whitener used for creaming coffee, tea, cocoa, and hot chocolate. It is marketed as a retail item that is ultra-high-temperature (UHT) processed and

Table 17.9. Comparison of cream products.

% Composition	Heavy cream	Light whipping cream	Light cream	Half and half
Fat range allowed[1]	Approximately 36	30–36 fat	18–30	10.5–18
Typical fat[2]	37	30.91	19.31	10.5
Typical solids nonfat[2]	5.29	5.59	6.94	7.93

[1]Values from Title 21, United States Code of Federal Regulations
[2]Values from USDA Nutrient Database http://www.nal.usda.gov/fnic/foodcomp/search/

packaged in dairy cartons similar to cream. As a food service item, it comes in individual size (portion-controlled, 0.5 to 0.75 fl oz) cups. The packages contain ultrapasteurized product for extended shelf life under refrigerated storage and brief exposure to room temperature during hospitality conditions. In view of the exposure to the acidic coffee environment, normal cream products tend to break down and curdle (feathering effect). Chelating agents and buffering salts such as sodium citrate or phosphate buffering salts are added to half and half to ensure stability in hot coffee. In the manufacture of half and half, all of the liquid ingredients are blended, and then dry milk is incorporated through a powder funnel or other blender to standardize to the correct fat and solids levels.

Fluid Non-Dairy Creamer/Whitener

Liquid non-dairy whiteners simulate coffee cream. They are an oil-in-water type of emulsion in which the oils substitute for the milk fat of cream. The emulsion is designed to deliver a creamy body and flavor.

Non-dairy creamer is packaged in dairy cartons for the retail market. As a food service item, it comes in individual size (portion-controlled, 0.5 to 0.75 fl oz) cups, similar to half and half. The packages contain ultrapasteurized product for extended shelf life under refrigerated storage or ambient storage if packaged aseptically. Normal cream products tend to curdle (feathering effect) during exposure to the acidic coffee environment. Chelating agents and buffering salts such as sodium citrate or phosphate buffering salts are added to both dairy and non-dairy creamers to develop their stability in coffee.

Some liquid whiteners are freeze-thaw stable; they are kept frozen for extended shelf life but are thawed prior to use. To improve their convenience, most non-dairy creamers now are long-life product manufactured by treating the liquid whitener at sterilization temperature-time combinations and packing the product aseptically. The high heating regime employed in aseptically packaged whitener can cause Maillard browning, giving the product an undesirable dark color. Therefore, no corn syrup is used in the formulation to avoid the interaction of reducing sugar with amino acids of protein in the formula.

A typical formula for aseptic liquid whitener is given in WO 2007/044782: 40% water, 12% to 15% palm oil, 3% to 6% sucrose, 1% to 2% sodium caseinate, 1% to 3% dipotassium phosphate, 0.02% salt, 1% to 5% emulsifier, 0.02% to 0.2% carrageenan and carboxy methyl cellulose, and 0.5% to 1% flavor (McKenna, Keller, and Streiff, 1988; Campbell and Morley, 1992). The product is said to be stable for six months at room temperature and for a year under refrigeration conditions.

Chocolate Milk and Breakfast Drinks

The manufacturing procedure for chocolate milk and breakfast drinks consists of blending the liquid ingredients and adding chocolate powder slowly into the liquid blend with

agitation at high speed for 15 minutes. The mix is then pasteurized and homogenized at conventional pasteurization temperatures for products with high solids, or, as is increasingly common, pasteurized on systems designed for extended-shelf-life (ESL) products using UHT (135°C to 150°C; 275°F to 302°F) for 2 seconds or longer) and dispensed through ultra clean fillers into sanitized packaging. A typical composition and suggested formulations of chocolate milk (full fat and low-fat) are shown in Tables 17.10 and 17.11, respectively.

A recipe for chocolate milk consists of 1.5% cocoa, 6% sucrose, 0.2% vanilla extract, 0.2% salt, 0.2% sodium alginate, and 91% homogenized milk (Tressler and Sultan, 1975). Another method involves preparation of chocolate syrup followed by blending in milk. Chocolate syrup is made by blending 16.2% cocoa, 80.6% sucrose, and 3.2% sodium alginate. The alginate is mixed with 5 times its weight of sucrose. The cocoa is mixed with the remaining four parts of sucrose. Four parts by weight of skim milk are slowly added with stirring, and the syrup is stirred to a smooth consistency. The liquid is heat treated at 66°C (150°F) and the mixture of sucrose and alginate is added slowly with stirring. The blend is further heated to 82°C to 88°C (180°F to 190°F) and held for 15 minutes. The syrup is quickly cooled to less than 10°C (less than 50°F) and used to flavor milk (Tressler and Sultan, 1975).

Sweetened and flavored milks are also popular for shelf-stable applications such as chocolate milk and breakfast drinks (Table 17.12). These drinks are processed using UHT temperatures and are dispensed under sterile conditions into sterile packages. Phosphates and citrates are commonly added to the blend to chelate minerals and improve protein stability due to the heat instability of the milk proteins during UHT

Table 17.10. Typical composition of chocolate milk and eggnog.

Component	Chocolate milk		Eggnog	
	Full fat	Low fat	Full fat	Low fat
% Milk fat	3.4	1.5	8.2	2.0
% Milk-solids-not-fat	8.3	8.7	8.6	9.0
% Sucrose	5.5	5.5	7.4	9.6
% Chocolate powder	1.3	1.3	0	0
% Eggnog base	0	0	4.9	1.1
% Stabilizer	0	0	0.4	0.3
% Total solids	18.5	17.0	29.5	22.0

Table 17.11. Suggested formulation of chocolate milk and eggnog.

Ingredient	Chocolate milk		Eggnog	
	Full fat	Low fat	Full fat	Low fat
% Skim milk	0	86.3	0	23.6
% 3.4% milk	88.4	0	62.6	58.8
% Nonfat dry milk, low heat	0.8	1.1	16.4	2.0
% 36.5% cream	1.1	3.0	2.5	0
% Cocoa powder	1.4	1.4	0	0
% Liquid sugar	8.3	8.2	11.1	14.2
% Eggnog base	0	0	7.0	1.1
% Stabilizer	0	0	0.4	0.3
% Total solids	100.0	100.0	100.0	100.0

Table 17.12. Ready-to-drink chocolate milk and breakfast drinks.

Percent Composition	Sweetened breakfast beverage	Sugar-free breakfast beverage	Chocolate milk
Water	14	21	1 (from sugar)
Fluid milk	70	70	90
Concentrate protein source (milk protein, whey)	2	2	0
Sucrose/lactose	8	0	6
Cocoa	1.5	1.5	2
Carbohydrate solids source (corn syrup solids, maltodextrin)	1	2	0
Added fat	1.5	1.5	0
Stabilizers, emulsifiers, fortifiers, high-intensity sweeteners, flavors)	2	2	2

Table 17.13. Typical formulation of cultured dairy products.

Percent Composition	Plain yogurt	Yogurt, blended style	Yogurt, FOB* style	Frozen yogurt, low-fat	Cultured buttermilk, nonfat	Sour cream
Milk fat	0.4–3.25	0.5–3.50	1.0–1.5	0–2.1	<0.5	18.0
Milk-solids-not-fat	12.5–14.0	10.0–12.0	12.7–13.0	10.1	10.3	8.5
Sucrose	0	6–10	0–6	12.6	0	0
Stabilizer	0–0.5	0.3–1.6	0.1–0.2	0.6	0	0.6
Corn syrup solids, 36 DE	0	0	0	5.4	0	0
Maltodextrin, 10 DE	0	0	0	3.6	0	0
Whey protein concentrate	0.8	0.8	0.8	2.4	0	0
pH	4.4	4.4	4.4	6.0	4.6	4.6

*Fruit on the bottom
Adapted from Chandan and Shahani (1995), Chandan and O'Rell (2006a,b)

processing and concerns about protein stability during storage. Ready-to-drink breakfast drinks are often fortified with minerals and vitamins.

Eggnog

The composition and formulation of eggnog is also shown in Tables 17.10 and 17.11. The processing procedure involves blending all the liquid ingredients; the stabilizer is dry blended with the eggnog base and added to the liquid blend and mixed thoroughly. The mixture is pasteurized at 79.4°C (175°F) for 25 seconds and homogenized at 57.2°C to 62.8°C (135°F to 145°F) and 3.5 MPa (500 psi) second stage, and 14 MPa (2,000 psi) first stage. The product is cooled to 1.7°C to 3.3°C (35°F to 38°F), packaged, and stored at 1.7°C to 4.4°C (35°F to 40°F).

Cultured Dairy Foods

Fermented dairy foods have constituted a vital part of the human diet in many regions of the world since time immemorial. They have been consumed ever since the domestication of animals. Milk is a normal habitat of a number of lactic acid bacteria, which causes souring of milk held at bacterial growth temperatures for an appropriate length of time. Depending on the type of lactic acid bacteria gaining entry from the environmental sources (air, utensils, milking equipment, milkers, cows, feed, etc.), the sour milk attains characteristic flavor and texture. A perspective on various fermented dairy ingredients including their applications is described in Chapter 13 of this book. The formulation of major cultured dairy foods is given in Table 17.13.

Cultured Buttermilk

Cultured buttermilk is obtained from pasteurized skim milk or part-skim milk cultured with lactic acid producing culture (*Lactococcus lactis* subsp. *lactis/cremoris/diacetylactis*) and aroma-producing culture *(Leuconostoc cremoris).* The term buttermilk is also used for the phospholipid-rich fraction obtained as a byproduct during the churning of cream in butter manufacture. Cultured buttermilk is a viscous, cultured fluid milk beverage possessing a characteristic pleasing aroma and flavor. It is usually produced in dairy plants that process milk and other fluid milk products. Buttermilk composition depends on whether it is nonfat or low fat. In general, the nonfat buttermilk contains 0.1% milk fat, 10% MSNF, and 0.18% salt. This formulation is achieved by blending 98.3% skim milk, 1.5% NFDM and 0.18% dairy salt. In contrast, low-fat buttermilk contains 1% milk fat and 9% MSNF. The process is similar to the outline given in Figure 17.3. The product is packaged in traditional milk cartons.

The manufacturing procedure consists of blending NFDM and salt into skim milk and mixing thoroughly. The blend is heat treated at 90.6°C (195°F) for 5 minutes and cooled to 22.2°C (72°F). The bulk starter is inoculated at the rate of 0.75% and ripening is carried out to 0.8% titratable acidity or pH 4.5, usually over 16 to 18 hours. The coagulum is broken and cooled to 1.7°C to 4.4°C (35°F to 40°F). Buttermilk is packaged and stored at 1.7°C to 4.4°C (35°F to 40°F).

Dairy plants can make their own buttermilk starter from frozen culture purchased from culture suppliers. Bulk starter is prepared from reconstituted NFDM. Using a powder funnel, 10.5% NFDM is dispersed into water. The mixture is pasteurized at 85°C (185°F) for 30 minutes, cooled to 22.2°C (72°F), and one can of frozen culture per the instruction of culture supplier is added. After mixing well, the mix is held at room temperature (22.2°C (72°F)) until the pH is 4.5 or titratable acidity is 0.83% to 0.87%. The incubation time is on the order of 16 to 18 hours. The bulk starter is cooled to 7.2°C (45°F) and used within four days.

In addition to its use as a beverage, buttermilk is also used in several foods for its functional attributes. In the baking industry, cultured buttermilk is used in pancakes, waffles, blintzes, breads, cookies, and cakes. It imparts a fluffy texture. Typical quality issues and their remedies in buttermilk and sour cream manufacture are given in Table 17.14.

Yogurt

Yogurt is a semi-solid fermented product made from a standardized mix by the activity of a symbiotic blend of *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB) cultures. The per capita consumption of yogurt has registered a dramatic rise in the United States; the consumption was 11.8 pounds in 2008 (IDFA, 2009). The increase in yogurt consumption may be attributed to its perceived natural and healthy image, providing to the consumer convenience, taste and wholesomeness attributes. Table 17.15 summarizes recent trends in consumption of refrigerated yogurt and fermented milk products in the United States. It is clear that yogurt is a very successful dairy food of modern times. Figure 17.4 illustrates various forms of yogurt in the U.S. market.

Dairy Ingredients. Yogurt is a Grade A product in the United States, and the milk that it is made from must come from FDA-supervised Grade A dairy farms and Grade A manufacturing plant as per regulations enunciated in the Pasteurized Milk Ordinance (PMO; United States Department of Health and Human Services, 2007). Yogurt is made

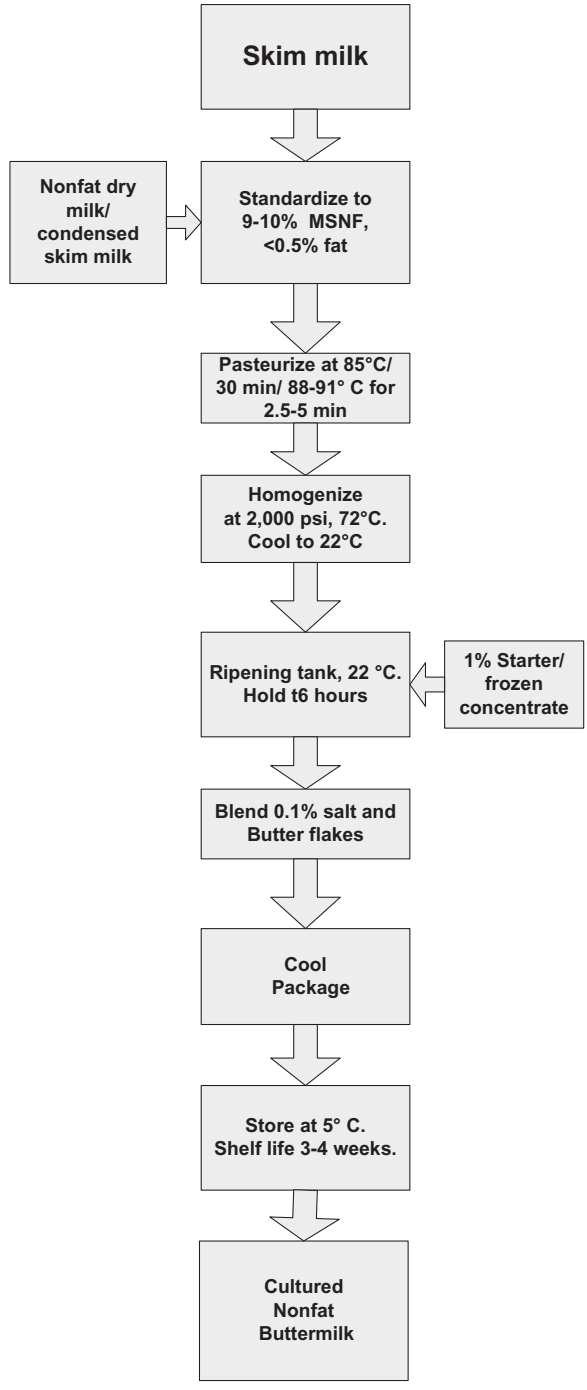

Figure 17.3. An outline for manufacturing cultured buttermilk.

Table 17.14. Some quality issues and their remedies in the manufacture of buttermilk and sour cream.

Defect	Possible cause	Remedy
Not enough flavor	Low citrate level in mix. Diacetyl destroyed	Incorporate 0.02%–0.05% sodium citrate to the mix before culturing. Cool rapidly after culturing. Agitate gently to avoid incorporating oxygen.
Green/yogurt flavor	Acetaldehyde accumulation	Avoid the use of *Lactococcus lactis* subsp. *diacetylactis*
Cardboard flavor	Copper contamination and exposure to sunlight/ fluorescent light	Avoid exposure to copper utensils. Protect packages from direct exposure to sunlight and source of UV light.
Yeast/cheese-like odor/flavor	Contamination with yeast and its growth	Check sanitation procedure. Avoid return milk in formulation
Rancid flavor	Lipase activity	Avoid mixing of raw milk and pasteurized product streams
Weak body	Insufficient heat treatment. Too low MSNF. Too severe agitation after culturing.	Ensure correct heat treatment: 85°C/30 minutes for buttermilk and 74°C/30 minutes for sour cream. Fortify with 0.5%–1% NFDM for buttermilk and 2%–3% for sour cream. Check stabilizer use. Use rennet in sour cream formulation.
Grainy texture	Acidity too high. NFDM is not dispersed properly.	Check processing procedure for acidity control. Ensure full dispersion of NFDM. Use in-line screen to remove large particles.
Chalky/powdery texture/mouth feel	Too much NFDM in formulation	Check the quality and quantity of NFDM

Adapted from Chandan and Shahani (1995)

Table 17.15. Annual total and per capita sales of refrigerated yogurt in the United States.

Year	Yogurt		Sour cream and dips		Buttermilk	
	Sales (million pounds)	Per capita sales (pounds)	Sales (million pounds)	Per capita sales (pounds)	Sales (million pounds)	Per capita sales (pounds)
2005	3,058	10.3	1,309	4.4	512	1.7
2006	3,301	11.0	1,256	4.2	504	1.7
2007	3,476	11.4	1,313	4.32	508	1.7
2008	3,599	11.8	1,274	4.19	547	1.8

Adapted from International Dairy Foods Association, Dairy Facts (2009)

from a mix standardized from whole, partially defatted milk, condensed skim milk, cream, and nonfat dry milk. It is common to supplement MSNF in the mix with NFDM. The FDA specification (CFR, 2009) calls for a minimum of 8.25% nonfat milk solids (SNF). However, the industry uses up to 12% SNF in the yogurt mix to generate a thick, custard-like consistency in the product. The milk fat levels are standardized to 3.25% for full-fat yogurt. Reduced fat yogurt is made from mix containing 2.08% milk fat. Low-fat yogurt is manufactured from mix containing 1.11% milk fat. Nonfat yogurt mix has milk fat level not exceeding 0.5%. These fat levels correspond to the FDA requirement for nutritional labeling of nonfat, reduced-fat and low-fat yogurt (Frye and Kilara, 2008). All raw dairy materials should be selected for high bacteriological quality.

Yogurt starters. A starter consists of food-grade microorganism(s) which, on culturing in milk, produce predictable attributes characterizing yogurt. The composition of

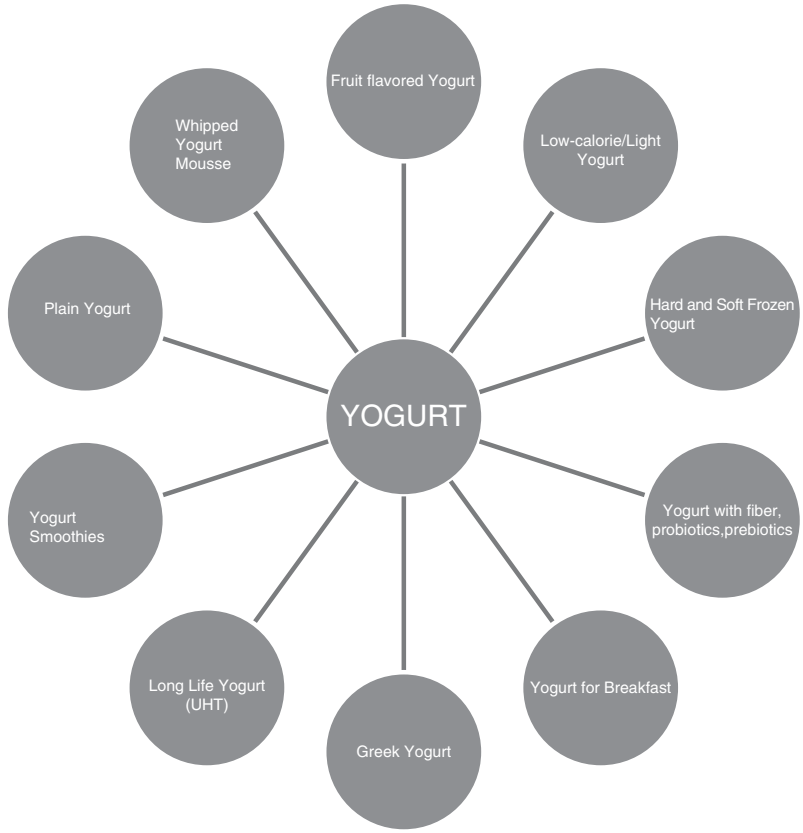

Figure 17.4. Types of commercial yogurt.

yogurt starter is shown in Table 17.16. Also, shown are some additional organisms found in yogurt or yogurt-like products marketed in various parts of the world. In some countries of Europe, *Lactobacillus bulgaricus* is replaced with *Lactobacillus lactis* to produce "mild" yogurt.

The influence of temperatures of incubation on the growth of yogurt bacteria is shown in Table 17.17. Acid production is normally used as a means of assessing the growth of yogurt culture.

Yogurt starter organisms display an obligate symbiotic relationship during their growth in the milk medium. The rate of acid production by a yogurt starter containing both ST and LB is considerably higher than by either of the two organisms grown separately. The acid-producing ability of yogurt culture in mixes containing 8% sucrose is fairly good.

Bacteriophages, virus-like microbes, kill bacteria by their lytic action. Phage infections lead to loss in rate of acid production. The yogurt fermentation process is relatively fast (two and a half to four hours).

Sweeteners. Nutritive carbohydrates (mainly sucrose) are frequently used in yogurt manufacture. High-intensity sweeteners (e.g., aspartame, sucralose, neotame, acesulfame K, etc.) are used to produce light yogurt containing about 60% of the calories of normal sugar-sweetened yogurt. Low levels of crys-

Table 17.16. Required and optional composition of yogurt bacteria.

Required by FDA standard of identity for yogurt	Optional additional culture used or suggested
Streptococcus thermophilus (ST)	*Lactobacillus acidophilus*
Lactobacillus delbrueckii subsp. *bulgaricus (LB)*	*Lactobacillus casei* *Lactobacillus casei* subsp. *rhamnosus* *Lactobacillus reuteri* *Lactobacillus helveticus* *Lactobacillus gasseri ADH* *Lactobacillus plantarum* *Lactobacillus lactis* *Lactobacillus johnsoni LA1* *Lactobacillus fermentum* *Lactobacillus brevis* *Bifidobacterium longum* *Bifidobacterium breve* *Bifidobacterium bifidum* *Bifidobacterium adolescentis* *Bifidobacterium animalis* *Bifidobacterium infantis* *Bifidus ActiRegularis*™

Adapted from Chandan (1999, 2004)

Table 17.17. Growth temperature profile of yogurt organisms.

Growth Temperature	*Streptococcus thermophilus* °C	*Lactobacillus delbrueckii* subsp. *bulgaricus* °C
Maximum	50	50–52
Minimum	20	15
Optimum	39–46	40–47

Adapted from Chandan and Shahani (1993)

talline fructose may be used in conjunction with aspartame and other high-intensity sweeteners to round up and improve the overall flavor of light yogurt. Up to 8% corn syrup solids are used in frozen yogurt. Commercial yogurts contain an average of 4.06% lactose, 1.85% galactose, 0.05% glucose, and pH of 4.40.

Stabilizers. Stabilizers (generally 0.5% to 0.7%) in yogurt produce smoothness in body and texture. Commonly used stabilizers include gelatin; starches; vegetable gums such as carboxymethyl cellulose, locust bean, and guar; seaweed gums such as alginates and carrageenans; and pectin. Whey protein concentrate is commonly used as a stabilizer, exploiting the water-binding property of denatured whey proteins.

Fruit Preparations for Flavoring Yogurt. The fruit preparations for blending in yogurt are specially designed to meet the marketing requirements for different types of yogurt. They are generally present at levels of 8% to 15% in the final product. Special fruit bases are designed for use in stirred yogurt. They generally contain 0.1% artificial flavor or 1.25% natural flavor, 0.1% potassium sorbate, and an appropriate level of coloring. The pH is adjusted to 3.8 to 4.2, depending on the particular fruit. Calcium chloride and certain food-grade phosphates are also used in several fruit preparations. The soluble solids range from 60% to 65% and the viscosity is standardized. To avoid unnecessary contamination of yogurt, aseptically packaged sterilized

fruit preparations are now preferred by yogurt manufacturers.

Yogurt Processing

Production of Yogurt Starters

Frozen culture concentrates are available from commercial culture suppliers. Many plants use frozen direct-to-vat or freeze-dried direct-to-vat cultures for yogurt production. However, for cost savings, large yogurt manufacturers prefer to make bulk starters in their own plant from frozen or freeze-dried bulk cultures. The medium for bulk starter production is antibiotic-free NFDM reconstituted in water at the 10% to 12% solids level. Following reconstitution of nonfat dry milk in water, the medium is heated to 90°C to 95°C (194°F to 203°F) and held for 30 to 60 minutes. The medium is then cooled to 43°C (110°F) in the vat.

The incubation period for yogurt bulk starter ranges from four to six hours, and the temperature of 43°C (110°F) is maintained by holding hot water in the jacket of the tank. The progress of fermentation is monitored by titratable acidity measurements at regular intervals. When the titratable acidity is 0.85% to 0.90%, the fermentation is terminated by turning the agitators on and replacing warm water in the jacket with iced water. Circulating iced water drops the temperature of the starter to 4°C to 5°C (39°F to 41°F).

Mix preparation. Yogurt mix is prepared from the ingredients by standardization of milk for fat and MSNF content. The addition of stabilizers (gelatin, starch, pectin, agar, alginates, gums, and carrageenans) and sweeteners impacts physical properties.

Heat treatment. Heat treatment at 85°C (185°F) for 30 minutes or 95°C to 99°C (203°F to 210°F) for seven to 10 minutes is an important step in manufacture. The heat treatment (a) produces a relatively sterile medium for the exclusive growth of the starter, (b) removes air from the medium to produce a more conducive medium for microaerophilic lactic cultures to grow, (c) effects thermal breakdown of milk constituents, especially proteins, releasing peptones and sulfhydryl groups which provide nutrition and anaerobic conditions for yogurt culture, and (d) denatures whey proteins of milk, thereby enhancing the viscosity, leading to a custard-like consistency in the product.

Physical changes in the proteins as a result of heat treatment have a profound effect on the viscosity of yogurt. Whey protein denaturation, of the order of 70% to 95%, enhances water absorption capacity, thereby creating a smooth consistency, high viscosity, and stability from whey separation in yogurt.

Homogenization. Homogenization is usually conducted by applying pressure in two stages. The first stage pressure, of the order of approximately 14 MPa (2,000 psi), reduces the average milk fat globule diameter size from approx. 4 µm (range 0.1 to 16 µm) to less than 1 µm. The second stage uses 3.5 MPa (500 psi) and is designed to break the clusters of fat globules apart to inhibit creaming in milk. Homogenization aids in texture development and alleviates the surface creaming and wheying off problems. The homogenized mix is brought to 43°C (110°F) by pumping through an appropriate heat exchanger. It is then collected in fermentation tanks.

Fermentation. Fermentation tanks for the production of cultured dairy products are generally designed with a cone bottom to facilitate draining of relatively viscous fluids after incubation. Using bulk starters at the 4% inoculum level, the incubation period is 2.5 to 3 hours at 43°C (110°F). During fermentation, lactose content of the mix is reduced by approximately 30%. However, a significant level of lactose (4.2%) survives in yogurt. Normally, the fermentation period is terminated by a temperature drop to 4°C

(39°F) as the yogurt mass is pumped through a heat exchanger. A texturizing cone is inserted in the pipe leading to the heat exchanger to smoothen the texture.

Manufacturing Procedures

Plain Yogurt. Plain yogurt is the basic yogurt style, and it forms an integral component of fruit-flavored yogurt. The steps involved in the manufacturing of set-type plain yogurt are shown in Figure 17.5. Plain yogurt normally contains no added sugar or flavors. It has a natural yogurt flavor for consumption as such or it can be flavored with other ingredients of the consumer's choice. In addition, it may be used for cooking or for salad preparation with fresh fruits or grated vegetables. In most recipes, plain yogurt is a sour cream substitute that provides fewer calories and a fat alternative.

Fruit-flavored yogurt. In addition to plain yogurt, Figure 17.5 also illustrates a manufacturing outline for fruit-on-the-bottom style yogurt, which includes the use of two-stage fillers. Typically, 59 ml (2 oz) of special fruit preparation is layered at the bottom of the cup, followed by 177 ml (6 oz.) of inoculated yogurt mix on the top. The top layer may consist of unfermented yogurt mix containing stabilizers, sweeteners, and the flavor and color indicative of the fruit on the bottom. After placing lids on the cups, incubation and setting of the yogurt takes place in the cups. When a desirable pH of 4.4 to 4.5 is attained, the cups are placed in refrigerated rooms with high-velocity forced air for rapid cooling.

Stirred style yogurt or blended yogurt is produced by blending the fruit preparation thoroughly in fermented yogurt base obtained after bulk culturing in fermentation tanks. Figure 17.6 illustrates the process flow.

Stabilizers, especially gelatin, are commonly used in this form of yogurt unless MSNF levels are relatively high (12% to 14%). In this style, cups are filled with an in-line blended mixture of yogurt and fruit. Several variations of this procedure exist in the industry. Fruit incorporation is conveniently effected by the use of a fruit feeder at the 8% to 15% level.

Aerated yogurt. Aerated yogurt resembles mousse in that the product acquires a novel foam-like texture. The aeration process is similar to the ice cream process, but the degree of overrun (extent of air content) is kept relatively low, 20% to 50%. Foam formation is facilitated by use of appropriate emulsifiers and the stability of foam is achieved by using gelatin in the formulation. After fermentation and cooling, aerated yogurt is produced with appropriate equipment (Oakes, Tanis, or Mondo), injecting a controlled volume of an inert gas (nitrogen) to create foam in the product. Nitrogen helps to control fat oxidative issues in the product. The amount of overrun is related to textural and mouth feel attributes of the product and is determined by the desired marketing requirements. In aerated yogurt, it is desirable to measure overrun to ensure uniformity of texture from day to day. The volume of yogurt in the cup also is related to the degree of overrun. Accordingly, overrun control ensures the correct weight of the product in the cup.

Yogurt-based salad dressings. Yogurt-based salad dressings contain salt, spices, and herbs such as dried onion, garlic, and parsley. Some yogurt dressings may contain honey, Dijon mustard, and celery seed. Yogurt dips may also contain onion, clam, cheddar, and blue cheese, depending on the product concept.

Heat-treated yogurt. The shelf life of yogurt may be extended by heating the yogurt after culturing to inactivate the culture and the constituent enzymes. Heating to 60°C to 65°C (140°F to 149°F) extends the shelf life to about 12 weeks at 12°C (54°F). UHT treatment and aseptic packaging ensures a shelf life that is even longer, even at room storage temperature. However, these

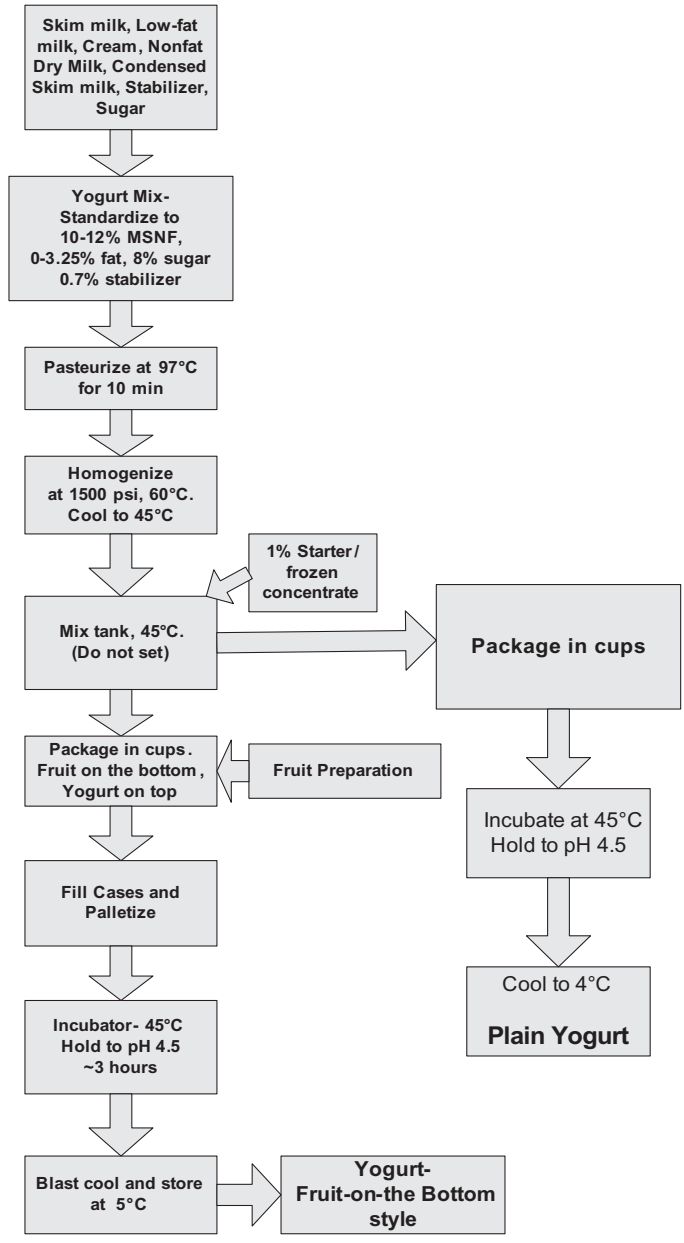

Figure 17.5. Process flow diagram for the manufacture of plain yogurt and fruit-on-the bottom style yogurt.

treatments destroy the live and active nature of yogurt, which may be an essential consumer attribute.

Greek yogurt. Greek-style yogurt is typically heavy bodied and viscous. It is produced by straining or centrifuging some of the whey from yogurt or by reconstituting dry or concentrated ingredients and culturing them to create a high-solids (22% to 26%) fermented product. The fat content can be

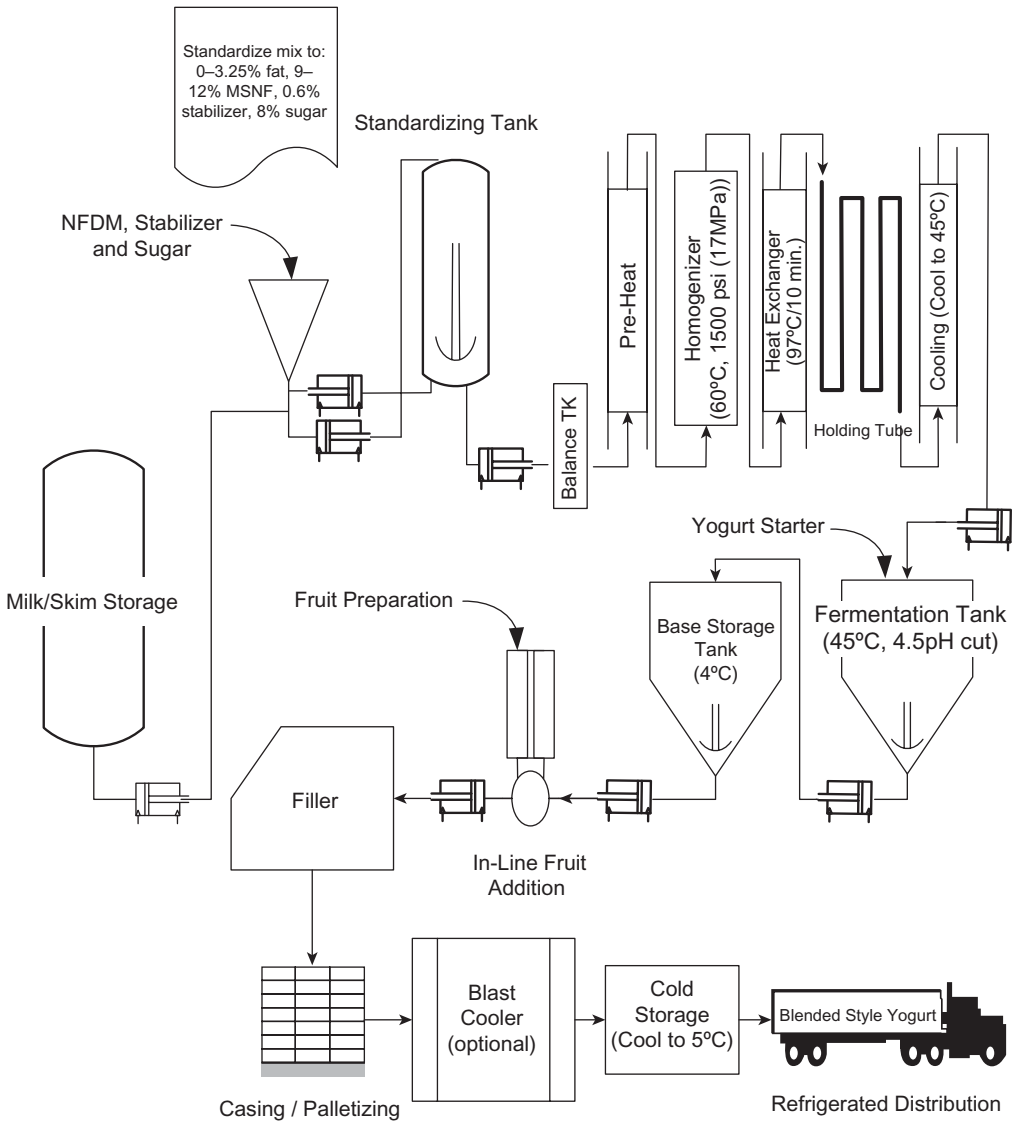

Figure 17.6. Flow sheet for the manufacture of blended (stirred style) yogurt. Chandan and O'Rell (2006).

standardized by adding pasteurized cream to the strained yogurt. Because the straining step removes some whey, which contains lactic acid, strained Greek-style yogurt tastes milder than regular yogurt. It contains more protein than regular yogurt because of the partial whey removal.

Yogurt beverages. Yogurt beverages, also called drinking yogurt, yogurt smoothies, and yogurt drinks, are low-viscosity yogurt made from low-solids mix. Another variation consists of blending yogurt with milk, water, and fruit juice, and subjecting the mixture to extra shear (homogenization) to reduce viscosity. The stabilizers are non-thickening type, such as high-methoxy pectin, gelatin, and modified starch, to impart smooth texture and control whey separation during the

shelf life. A recent trend is to include fructo-oligosaccharide prebiotics, such as inulin, and to fortify with a significant daily requirement of most vitamins and minerals.

Kefir is another fermented drink obtained by use of Kefir culture. It has a distinctive flavor, and most kefir products in the United States are sweetened with sugar and flavored with fruit juices.

Quality Control

For refrigerated yogurt and yogurt beverages, most spoilage organisms are yeasts and molds which are highly tolerant to low pH and can grow under refrigeration temperatures. If yeast contamination is not controlled, fungal growth manifests within two weeks of manufacture. To ensure maximum shelf life, several manufacturers use potassium sorbate to control the growth of yeasts and molds in the product.

Quality control checks (spot checking) should be performed on fruit preparations and flavorings to ascertain sterility and to eliminate yeast and mold entry via fruit preparation. Refrigerated storage of the fruit flavorings is recommended.

Quality control programs for yogurt include control of product viscosity, pH, flavor, body and texture, color, fermentation process, and composition. Product standards of fats, solids, viscosity, pH (or titratable acidity), and organoleptic characteristics should be checked regularly. Wheying off or appearance of a watery layer on the surface of yogurt is undesirable and can be controlled by judicious selection of effective stabilizers and proper processing conditions.

Yogurt products enjoy an image of a health-promoting food. The type of cultures and their viability as well as active status are important attributes from the consumer standpoint. Generally, at the time of manufacture, yogurt should contain not less than 100 million CFU/g. Assuming that the storage temperature of yogurt through distribution channels and in grocery stores is 4°C to 7°C (39°F to 45°F), a loss of one log cycle in culture viability is expected between manufacture and consumption. Therefore, at the time of consumption, yogurt should deliver at least 10 million CFU of live yogurt organisms/g of the product.

Sour Cream

Sour or cultured cream is manufactured by ripening pasteurized cream of 18% fat with mesophilic lactic acid culture (*Lactococcus lactis* subsp. *lactis/cremoris/diacetylactis*) and aroma-producing *Leuconostoc cremoris*. The culture used is identical to that used in cultured buttermilk, but in consistency sour cream is an acid gel with a butter-like aromatic flavor. Sour cream is used as a topping on vegetables, baked potatoes, salads, fruit, fish, and meats, and in soups and as a filling in cakes. It is an integral component of Mexican cuisine. Sour cream can be dehydrated by spray drying and used as an ingredient wherever its flavor is needed.

The composition of sour cream is 18.5% milk fat, 7.1% MSNF, 0.4% stabilizer, and 26% total solids. It is considered desirable to supplement cream (which contains 6.8% MSNF) with nonfat solids to increase the viscosity of the finished product. The formulation consists of 50.9% skim milk, 48.6% cream (40% fat), and 0.5% stabilizer. It is customary to add a small quantity of rennet. More flavor can be generated after culturing if 0.15% to 0.2% sodium citrate is incorporated in the sour cream mix.

Sour cream is manufactured by blending the stabilizer in a portion of approximately 10% skim milk through a powder funnel (Figure 17.7). The cream is then added along with the remainder of skim milk to flush the line. The mix is heat treated at 87.8°C (190°F) for 3 to 5 minutes, homogenized at 71.1°C (160°F) and 17.2 MPa (2,500 psi) (single stage), cooled to 22.2°C (72°F), and pumped to a cone vat. In general, sour cream

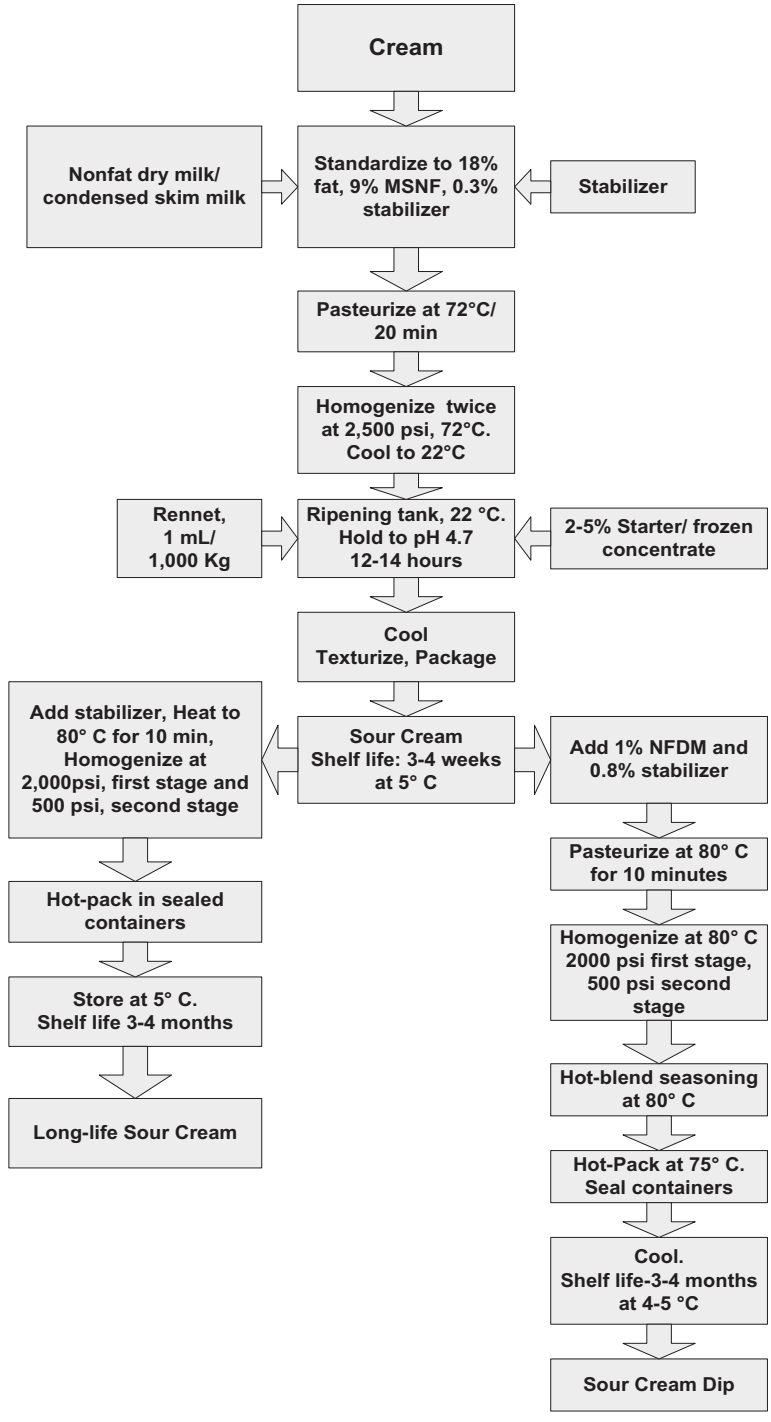

Figure 17.7. Diagrammatic scheme for sour cream and dip manufacture.

of higher viscosity is obtained by a long-hold pasteurization treatment as compared to a high-temperature-short-time (HTST) pasteurization procedure. However, the stabilizers compensate for the difference. The bulk starter (approximately 1% to 3%) is inoculated along with diluted rennet. After mixing for 20 minutes, the mix is held at 22.2°C (72°F) until a pH of 4.5 or titratable acidity of 0.75% is reached. It is further held for 20 minutes and the curd is broken by starting agitator and cooled to 18.3°C (65°F). Sour cream is packaged using a positive drive pump fitted with a sour cream valve for smoothing the texture. The product is held at 1.7°C (35°F) for 24 to 72 hours before shipping.

The manufacturing procedure given in Figure 17.7 is a general procedure. By running the mix through the homogenizer twice, it is possible to produce sour cream of increased viscosity. Cultured cream can be manufactured in bulk fermentation, followed by cooling and packaging in large containers for retail market. Individual portions (1- to 2-oz) of sour cream are manufactured by filling individual containers with sour cream mix seeded with sour cream culture, followed by incubation and cooling. A heavy-bodied product is formed on setting. Factors affecting viscosity of sour cream include acidity level, mechanical agitation, heat treatment, MSNF content of the mix, rennet addition, and homogenization treatment.

Long-life sour cream is obtained by a hot-pack process which destroys the culture cells and the enzymes present in the finished product. Packaging in a plastic or metallic container with a hermetically sealed lid further ensures prevention of recontamination of microorganisms and protects from oxidative deterioration of milk fat in the finished product.

Artificial or Filled Sour Cream

Artificial or filled sour cream is a product in which part or all of the milk fat has been replaced with vegetable oil or fat. It is an alternative for consumers concerned about saturated fats. Artificial sour cream can be manufactured by the method given in Figure 17.7, except that artificial cream is used in place of real cream. An artificial cream may be prepared by emulsifying a suitable vegetable oil, flavor and color, emulsifier, and stabilizer in skim milk by homogenization.

Sour Cream Dips

Party dips based on sour cream are made by blending appropriate seasoning mixes into cultured cream. By packaging them under refrigerated conditions, the products have a shelf life of two to three weeks under refrigerated storage. However, for a shelf life of three to four months, the process shown in Figure 17.7 is used. To increase body and stability, 1% to 2% NFDM and 0.8% to 1% stabilizer are incorporated in sour cream at 80°C (176°F). The mixture is heat-treated by holding it for 10 minutes and homogenizing at 17.2 kPa (2,500 psi) to create a smooth texture. The seasonings are blended at this stage while the mix is still at 80°C (176°F), followed by hot-packing in sealed containers. Upon cooling and storage at 5°C (41°F), partial vacuum inside the container assists in the prevention of oxidative deterioration to yield an extended shelf life of three to four months.

Sour Half and Half

Sour half and half is similar to sour cream except that its fat content is only 10.5%; thus, its lower fat and calorie content appeal to certain consumers. Sour half and half is manufactured from a mix containing 10.5% to 11% fat. To compensate for the reduction in solids due to the lower fat level, it is customary to increase the MSNF level to 10% to 12% MSNF with NFDM. The mix is then processed along the same lines as sour cream, as shown in Figure 17.7.

Salad Dressing

Salad dressing can be prepared with sour half and half as a base, which subsequently may be blended to produce a distinctive creamy dressing. This refrigerated dressing contains 50% to 75% fewer calories than conventional salad dressings, but has a comparable flavor and texture. The reduction in calories is primarily attributed to a lower fat level of 10.5% in sour half and half dressing as compared to 30% to 80% oil in regular dressing.

Refrigerated and Shelf-Stable Ready-to-Eat Desserts/Snacks

Dairy desserts are thickened and set product made by adding sweeteners; varieties of starch, rice, or rice powder; tapioca granules; gelatin; seaweed extracts such as alginates, carrageenan, and other hydrocolloids; tapioca; or eggs to milk or skim milk. Crushed nuts and dry fruits also may be incorporated to add variety of texture and flavor. Dairy desserts may be used as snacks or desserts. A detailed discussion on dairy desserts is given elsewhere (Chandan and Kilara, 2008).

This section deals with the refrigerated and shelf-stable category that includes pudding, custard, mousse, crème brulee, and cheesecake. The final texture in most dairy desserts is derived from the interaction of milk casein with carrageenan along with viscosity that can be generated from modified starch. The texture varies from soft, creamy, and spoonable to gelled and firm. Flan and some puddings may be firm enough to be molded in a packaging cup with a sauce or syrup at the bottom. After removal from the cup and retrieval in a dessert plate, it is consumed with the sauce flowing down from the top. Generally, these products are pH-neutral. Occasionally, they are directly acidified or cultured. The custards and puddings may be prepared or baked in a pie crust to make pies such as tarts, cheesecakes, key lime pie, or cream pie. The advent of shelf-stable, ready-to-eat puddings in single-serving containers gives the consumer convenience and portability. In general, these dairy desserts do not have a standard of identity defined by the FDA.

Dessert puddings include bread pudding, carrot pudding, chocolate pudding, vanilla, pudding, butterscotch pudding, pistachio pudding, plum pudding (Christmas pudding), fruit pudding, rice pudding, tapioca pudding, date and toffee pudding, pie fillings, blancmange, custard, junket, mango pudding, parfait, and mousse. Puddings in frozen form are exemplified by frozen custard and pudding pops.

Market Value

The market for refrigerated dairy desserts is fairly significant in European countries. However, in the United States, pudding is not perceived by the consumer as being as healthy as yogurt. In reality, pudding is also a dairy category that is wholesome, nutritious, low in fat, and high in calcium (Smith, 2003). In 2004, the sales of refrigerated pudding, mousse, gelatin, and parfait brands were $570.4 million with a change of –0.1% from the previous year. Total sales for shelf-stable pudding and gelatin brands were $283.6 million and the sales growth vs. that of the previous year was 7.7%. (AllBusiness, 2007).

Types of Puddings and Dairy Desserts

Various types of puddings and dairy desserts are classified in Figure 17.8. The broad classification shows some examples of common names of desserts and snacks. Recent trends include more technically sophisticated products.

- Layered mousses
- Pudding with swirls
- Vertically and horizontally layered products
- Combinations of mousses, gelatin desserts, flans, cakes, and puddings.
- Healthy and indulgence products

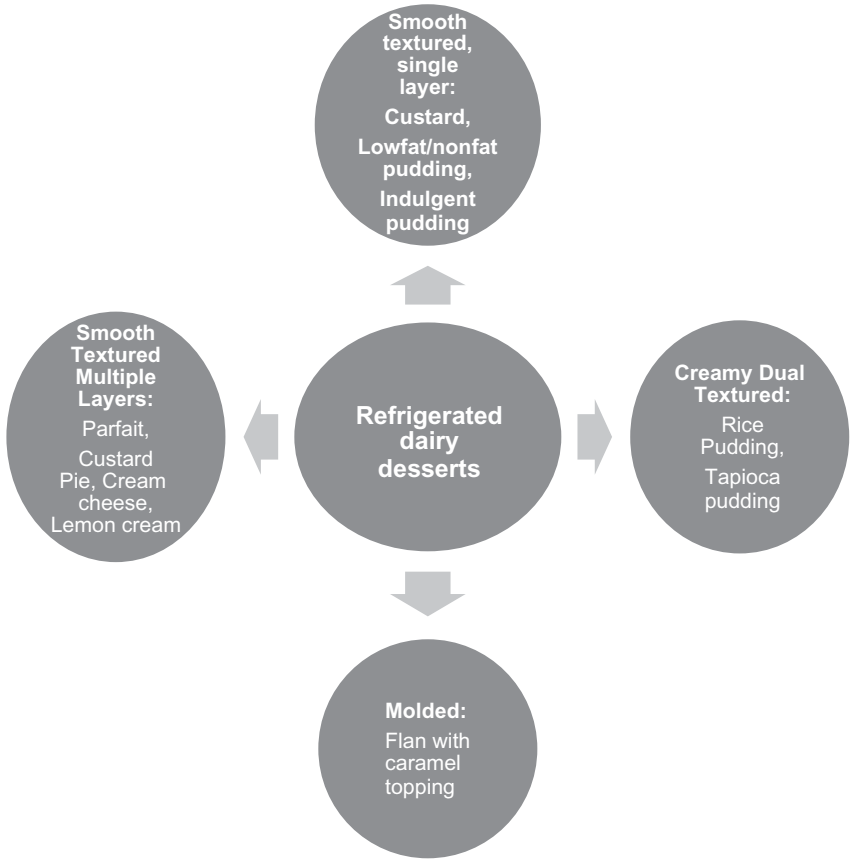

Figure 17.8. Major classification of puddings and dairy desserts.

Ingredients

The milk fat and MSNF in pudding mix composition is generally standardized from whole, partially defatted milk, nonfat dry milk, and/or condensed skim milk and cream. Cream is used to standardize the fat content of the pudding mix, especially in indulgent versions of puddings. In some cases, 0.5% to 1% WPC (34% protein level) may be used in a pudding mix and the rest of the milk solids are derived from nonfat dry milk.

Milk protein concentrate (MPC) is a functional ingredient that raises the protein level of the mix, but the main reason for its use is to reduce the lactose content of the mix to produce a low-carbohydrate/-lactose product. The lactose level can be significantly reduced, as much as 70%, by judicious use of lactose-reduced MPC and high-protein WPC in the formulation, replacing milk and NFDM.

Pudding may be low fat or nonfat corresponding to FDA requirement for nutritional labeling (Frye and Kilara, 2008). In some puddings, milk fat is replaced with vegetable fat.

Typical puddings contain approximately 13% to 16% sugar equivalent. The sweetener most commonly used in the industry is sucrose in either liquid (65% to 67% total solids) or granulated form. The consistency of pudding is better when sugar is added to the milk rather than into the set base. Generally, frozen pudding or custard produc-

ers use regular corn syrup or high fructose corn syrup.

Puddings made with non-nutritive sweeteners are labeled as "no sugar added." They are attractive to consumers interested in reducing intake of carbohydrates or calories. When replacing sugar with high-intensity sweeteners, it is necessary to incorporate bulk agents such as maltodextrins or polydextrose. The level of aspartame, sucralose, and acesulfame potassium in pudding is of the order of 0.14%, 0.03% to 0.05%, and 0.03% to 0.05%, respectively. The current trend in the use of high-intensity sweeteners is to blend two or more sweeteners to optimize the sweet flavor profile. More recently, natural high-intensity sweeteners have become available. Stevia products are being used in soft drinks. In certain sweeteners, dry stevia extract (Reb A) along with erythritol, a polyol, is being used. Agave syrup, claimed to be a low-glycemic-index sweetener, is also available for use in foods.

Native and Modified Starch

Puddings can be formulated and processed for refrigerated or ambient distribution and storage. Refrigerated pudding is processed in conventional heat-treatment systems and packaged in non-aseptic conditions and must be refrigerated at all times. However, most pudding products on the market are heat sterilized and aseptically packaged and are marketed under ambient storage conditions.

Originally, puddings and custards were produced from milk and corn starch by batch-processing in vats. Corn starch gave a typical consistency and texture to the pudding and a starchy eating quality. Modified waxy maize starch produces a smooth texture, imparts stability to the product even at low temperature, and provides temperature and shear resistance. Modified tapioca improves spoonability.

At present, commercial puddings are not made with native corn starch because of stability issues. Native starch does not survive ultra-high heat treatment. By selecting available modified starches, both shelf-stable and refrigerated puddings have a built-in freeze-thaw stability to provide consumers with extended shelf life with frozen storage.

Modified starch is the basis of texture generation in pudding products. It is used at the 3% to 6% level in pudding formulation. Lower levels are employed to create a less gelled structure, whereas higher levels yield a firmer gelled structure.

Modified starches are mainly derived from corn starch, tapioca, and waxy maize starch. From the pudding manufacture standpoint, there are two classes of modified starch. One class is cross-linked and stabilized starch, which is basically used for creating viscosity. Selection of the appropriate modified starch to optimize the swelling property and maximize the viscosity should be based on the pudding process and packaging conditions. The second class of starches encompasses converted starch obtained by treatment with acid, enzymes, or certain chemicals. Acid-converted starches derived by reaction with hydrochloric or sulfuric acid impart mouth feel enhancing properties, whereas enzyme-converted starches produce specialty maltodextrins with mouth-coating properties. Oxidizing agents such as potassium permanganate or hydrogen peroxide also produce converted starches to manipulate mouth feel characteristics. These starches create special mouth-coating attribute to enhance the creamy texture of pudding. A combination of these treatments may be applied.

It is important to select the type of starch according to the pudding process. For kettle or batch process, it is desirable to select less cross-linked starch. Starch with lower cross linking becomes overcooked at the higher temperatures used in UHT processing, leading to lower viscosity. In general, highly cross-linked starch is optimal for use in UHT processing.

All modified starches are pre-gelatinized. For use in dry instant pudding mixes, pre-gelatinized, cross-linked modified starch is subjected to cooking, followed by spray drying or drum drying. An instant gel is formed on contact with cold or ambient-temperature water. Starch upon proper cooking becomes soluble and loses the birefringence property (Rapp, 1986). Chemically modified starches are described in the U.S. CFR, Title 21, Section 172.892.

In the manufacture of rice or tapioca pudding, starch is generated *in situ* by the rupture of rice grains or tapioca during the cooking process. In general, stabilizers improve consistency and build viscosity, minimize whey separation and bind free water, and maintain the gel structure after pumping, mixing, and cooling.

The stabilizer system used in pudding preparations is generally a combination of starch and carrageenans. However, to build special properties, various vegetable stabilizers may be employed. Their ratios as well as the final concentration (generally 0.05% to 3%) in the product are carefully controlled to obtain the desirable effects.

During processing, the incorporation of the stabilizer should take place with strong agitation resulting in complete dispersion and a uniform suspension. To minimize potential lumps or "fish eyes," it is best to disperse the stabilizer in granulated sugar or NFDM during addition. Once dispersed in the mix, it is necessary to continuously agitate it to keep the stabilizer in suspension until it is fully hydrated while receiving proper heat treatment.

In pudding manufacture, carrageenans are commonly used as a thickener and stabilizer. They complex with milk proteins to form different types of gels. κ-carrageenan is generally used at 0.10% to 0.15% and ι-carrageenan concentration varies from 0.09% to 0.11%. The choice of carrageenan is based on whether the product is cold filled or hot filled. Several American pudding manufacturers hot fill their pudding products. κ-carrageenan gives a brittle, thick gel, whereas ι-carrageenan produces a soft gel which, on cold filling, gives a very slick and smooth texture. A combination with modified starch imparts a more creamy impression in the mouth.

In flan-type products, carrageenan facilitates gel formation and assists in unmolding of the product. In creamy products, it assists in providing a desirable consistency and thixotropic behavior.

Both algin and sodium alginate are heat stable and promote stabilization of the gel by complex formation with Ca^{+2} and casein. Pectins are occasionally used alone or in combination with other hydrocolloids to stabilize the structure of pudding. Very small amounts (0.07% to 0.15%) modify the consistency of the milk gel, making it stiffer and preventing any syneresis that might arise during handling, transportation, and distribution. Low-methoxy pectin retains the whey in a very flexible network that is formed in reaction with calcium ions present in the pudding.

Guar gum can be used in stabilizer systems for refrigerated or frozen pudding. Guar gum is non-gelling and is used mainly as a viscosity builder, stabilizer, and moisture-binding agent.

In some formulations, certain calcium-interacting gums produced by fermentation processes are used. Gellan gum is sensitive to calcium ions of milk, thereby forming gels similar to alginates. The usage level is 0.05% to 1.5%. The texture achieved is not quite identical to that obtained with starches.

Carboxy-methyl cellulose is readily soluble in either hot or cold water and is effective at high processing temperatures. By judicious combination of various hydrocolloids, it is possible to produce moldable pudding which retains its shape after removal from the cup.

Polyphosphates are used to control the degree of protein aggregation induced by

heat treatment exceeding 129.4°C (265°F) necessary for sterilizing the pudding mix. The protein aggregation is more noticeable in low-fat to nonfat pudding and is characterized by a white speckled and translucent appearance accompanied by chalky mouth feel. These attributes are considered defects in the product. The protein aggregates are of the order of 40 micrometers in size. The phosphate mixture comprises of equal weights of tetra sodium pyrophosphate and disodium dihydrogen pyrophosphate (also called sodium acid pyrophosphate) at a level of 0.05% to 0.5% by weight of pudding (Leshik, 1993).

Emulsifiers aid in the dispersion and mixing of dry ingredients and formation of a relatively firm and smooth texture, especially if milk fat is replaced with vegetable oil or fat. More common emulsifiers consist of acetylated monoglycerides, propylene glycol monoesters, glycerol lacto palmitates, and sodium stearoyl-2-lactylate. Most emulsifiers are used at low levels (0.02% to 0.08%). The level is raised to 0.15% when milk fat is replaced with vegetable fat.

Salt and egg (white and yolk) are commonly used in pudding formulation. Salt is used to round off the overall flavor and is generally used at a level of 0.20% to 0.30%. Egg yolk (frozen, sugared) imparts a characteristic flavor as well as a source of lecithin emulsifier. Liquid pasteurized whole egg is used in certain rice puddings.

Cocoa powder of 22% to 24% fat is used at the 1.5% to 2.5% level in pudding manufacture. In addition, chocolate liquor at the 1.5% to 2% level aids in improvement of chocolate flavor.

Vanilla is a very popular flavor of commercial pudding. Artificial vanilla flavor is prepared from synthetic methyl vanillin. In pudding manufacture, vanilla (2 times) is generally used at a level of 0.25% to 0.30% in vanilla pudding and at a level of 0.10% to 0.15% in chocolate pudding.

Butterscotch may be procured as liquid butterscotch topping or as dry butterscotch chips. Butterscotch topping is generally used at the 9% to 14% level.

Refrigerated Ready-to-Eat Pudding Using Starches

Tables 17.18 and 17.19 show composition and formulation of typical refrigerated puddings.

Kettle/Batch Process (Hot Pack)

A general manufacturing procedure for (nonsterile) refrigerated milk-based pudding involves blending dry ingredients in the mixture of milk and cream in a kettle. The color solution is boiled and added along with flavor during heating to 65.6°C (150°F). The mix is pumped to the processor for pasteurization; heat treatment of no more than 68.3°C (155°F) in the processor and holding time of 30 minutes are needed to meet pasteurization requirements. The pasteurized product is pumped to the pudding thermutator or

Table 17.18. Typical composition of refrigerated milk-based pudding.

% Component	Vanilla	Light chocolate	Dark chocolate
Milk fat	3.50	3.50	3.50
Milk-solids-not-fat	8.25	7.50	7.50
Sucrose	14.75	16.00	16.00
Modified starch	5.80	5.70	5.10
Vanilla	As needed	As needed	As needed
Color	As needed	—	—
Cocoa	—	1.40	2.50
Total solids	33.30	34.10	34.60

Adapted from Chandan (1997)

Table 17.19. Formulation for 1,000-pound batch of refrigerated dairy pudding.

Ingredient	Pounds		
	Vanilla	Light chocolate	Dark chocolate
Whole milk	750	732	726
Cream, 40% fat	25	25	25
Nonfat dry milk, low heat	18	13	13
Sucrose	148	160	160
Modified starches	58	57	51
Flavor and color	As needed	0	0
Cocoa	0	14	25

Adapted from Chandan (1997)

scraped-surface heat exchanger (SSHE). A converted ice cream freezer (without the cooling system) also has been used by connecting a steam supply in the jacket. The temperature of the product is raised to 90.6°C to 93.3°C (195°F to 200°F) with steam pressure.

The product is then run from the SSHE to the hopper of the packaging assembly via a holding tube so that temperature of the packaged product in the cup is 73.9°C (165°F). The lids are applied and the cups are inverted in the wire cases. Adequate interspaces are allowed for proper cooling and the cups are held at room temperature for 10 to 15 minutes for pasteurization of the interior of the cup and lid before cooling. They are then transferred to a freezer at −22°C (−8°F) to cool the product to 10°C (50°F). To facilitate cooling within an hour, it is essential to blow cold air onto the cases. After the product is at 10°C (50°F), the wire cases are held at a temperature below 7°C (below 45°F) overnight before the cups are transferred to shipping cases. The product is released for shipment if it meets the standards. The shelf life of this pudding is 45 to 60 days at 5°C to 7°C (41°F to 45°F).

Sterilized and Aseptically Packaged Starch-based Milk Pudding

A suggested formula for sterilized and aseptically packaged starch-based milk pudding is given in Table 17.20. Figure 17.9 shows the general steps for the manufacture of shelf-stable and extended shelf-life refrigerated pudding.

The sterilized pudding process requires more rigorous heat treatment (Chambers and Nelson, 1993). Direct heating systems use live stem injection or infusion into the product. The ultra-high temperature (UHT) processing is conducted in appropriate equipment to accommodate increases in viscosity during heat treatment of the mix. It is common to subject the mix to a preheating temperature range of approximately 49°C (120°F). In the subsequent step, the preheated mix enters the UHT system where the temperature is raised to 135°C to 148.9°C (275°F to 300°F). The mix then enters a holding tube where it is held for the appropriate time (2 to 3 seconds) to ensure cooking of the pudding and effective microbial kill to achieve sterility of the product. Thereafter, the cooked pudding is cooled to a temperature range of 26°C to 30°C (79°F to 86°F) and stored in an aseptic vessel followed by packaging in an aseptic environment.

Pudding may be processed without the use of a scraped surface heat exchanger if the viscosity remains relatively low after aseptic heat treatment (Joseph et al., 1988). Such a product is formulated by using 73.2% skim milk, 17.43% sucrose, a low starch level (2.8% modified waxy maize starch), 5.62% vegetable fat containing hydrogenated

Table 17.20. Suggested formulation for long-life, aseptically packaged vanilla, chocolate, and butterscotch pudding.

Percent Composition	Vanilla	Chocolate	Butterscotch
Milk, 3.3% fat	63–68	56–63	63–68
Sugar, granulated	13–16%	12–17	7–10
Cream, 36% fat	7–8%	3.5–4.5	3–6
Water	5.5–7.0	10–14	2.5–5.0
Cocoa powder	0	1.5–2.5	0
Chocolate liquor	0	1.5–2.0	0
Butterscotch topping	0	0	9–14
Cross-linked waxy maize starch	3.5–4.5	3.5–4.5	3.5–4.5
Vanilla extract, 2×	0.25–0.30	0.10–0.15	0
Salt	0.15–0.25	0.20–0.30	0.25–0.35
κ-carrageenan	0.10–0.20%	0.10–0.15	0.25–0.35
ι-carrageenan	0.07–0.14%	0.09–0.11	0
Sodium stearoyl-2-lactylate	0.03–0.05	trace	0.03–0.05
Color	As needed	0	0.02–0.04
Butterscotch flavor	0	0	0.03–0.05
Calcium oxide	0	0	0.015–0.025

Adapted from Rapp (1986)

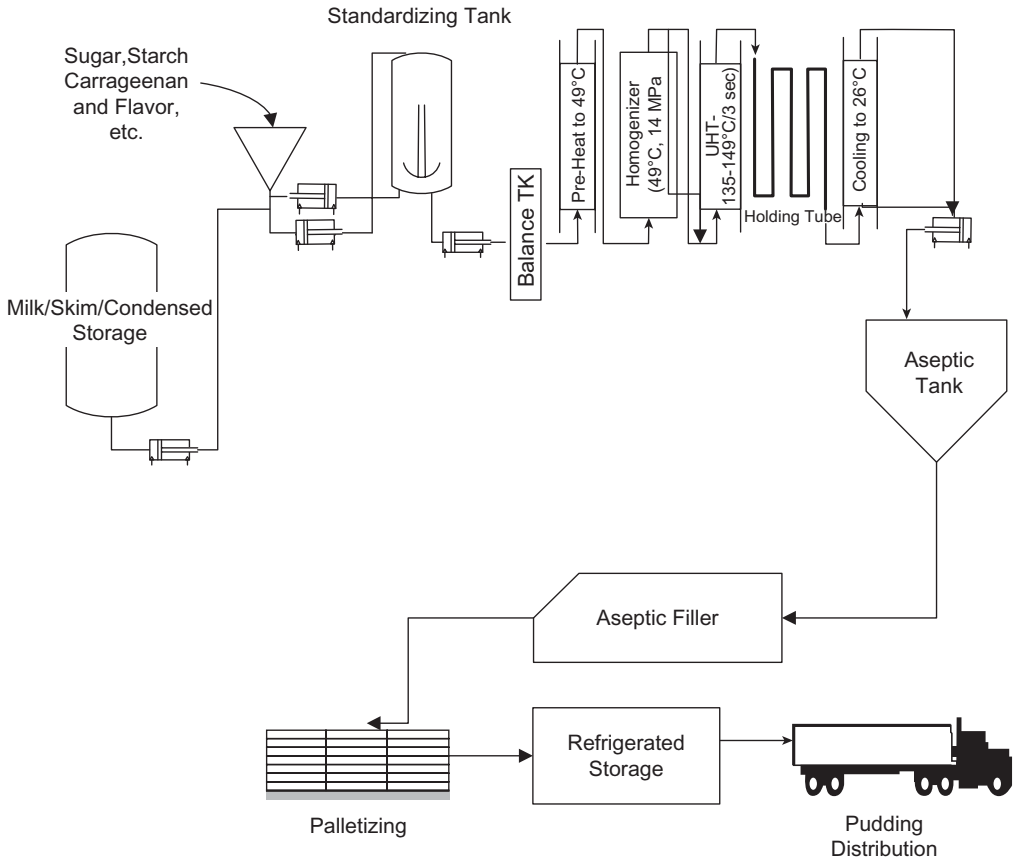

Figure 17.9. Manufacturing outline for the manufacture of aseptic, smooth pudding. Chandan and Kilara (2008).

coconut and palm kernel oils with a melting point of 38.9°C (102°F), 0.4% vanilla extract flavor, 0.2% salt, 0.15% color/flavor, and 0.2% sodium stearoyl 2-lactylate to develop low on-line viscosity after the cooking and sterilizing stage. This product develops optimum viscosity only after refrigeration of the packaged cups.

Single-serve containers of pudding are packaged in a form-fill system in which plastic containers are thermoformed or molded from packaging materials such as high polystyrene and filled with sterile pudding. Subsequently, the cups are sealed with lids cut from flexible lid stock. The lid material may be foil-laminated polyester with a heat-sealable coating, which makes a good seal after heat is applied. All of these steps are carried out in an enclosed aseptic system. If preformed cups and lids are used, they are unscrambled and sterilized with superheated steam, hydrogen peroxide, ultraviolet light, or high-intensity light. The flexible lid stock also must be sterilized. The fill temperature is 26°C to 30°C (78°F to 86°F).

The product filling temperature characterizes a refrigerated dairy dessert. Hot-filled desserts such as flan are packaged hot into cups at a temperature above the gelation point of the gelling agent (for example, carrageenan) used. As the product cools, it forms the texture of a firm gel. On the other hand, to obtain a minimal change in the post-packaging texture, the processed product is cooled under shear conditions as the gelling agent is setting up the gel network. Cold-filling gives a creamy texture without the appearance of a gel. Puddings and mousses are, therefore, packaged at below 15°C (below 59°F).

In some cases, manufacturers do not have rigorous aseptic conditions for producing the truly aseptic product that is suitable for ambient storage of the pudding product. However, they can package sterilized pudding in clean containers in a clean environment to produce extended shelf life (ESL) refrigerated pudding.

A general processing procedure for aseptically processed dairy pudding consists of dry blending sugar, nonfat dry milk, corn syrup solids, guar gum or other stabilizers, carrageenan, salt, and modified starches, and adding the dry blend slowly with agitation to water or liquid milk at 26.7°C (80°F). The mixture is heated to 48.9°C (120°F). Next, a mixture of vegetable oil (if used) and emulsifier is incorporated at 48.9°C (120°F) in the aqueous blend, followed by addition of color and flavor. After mixing thoroughly, the mix is homogenized at 48.9°C (120°F). It is then cooked at 121°C to 148.9°C (250°F to 300°F) through sterilization equipment and held for 2 to 3 seconds, as recommended by the equipment manufacturer. The pudding is cooled to 26.7°C (80°F) and held in aseptic storage. The product is packaged in aseptic packaging systems.

Vegetable Fat Pudding

Table 17.21 shows a formulation for pudding in which milk fat has been replaced with an appropriate vegetable fat. This product is produced by a process similar to other aseptically packaged products. The vegetable fat is emulsified by homogenization in the presence of a functional emulsifier.

Table 17.21. Typical formulation of aseptically packaged vegetable fat pudding.

% Composition	Vanilla	Chocolate
Sucrose	14.77	14.77
Nonfat dry milk, low heat	5.18	5.18
Corn syrup solids, 36 DE	2.46	2.46
Guar gum	0.12	0.12
Carrageenan	0.05	0.05
Cocoa	0	2.92
Salt	0.09	0.09
Modified starch	3.12	3.00
Emulsifier	0.13	0.13
Vegetable fat	6.61	6.60
Water	67.47	64.68
Color	As needed	—
Flavor	As needed	—

Adapted from Chandan (1997)

Table 17.22. Typical formulation of fat-free and low-fat pudding.

% Composition	Fat free, chocolate	Fat free, vanilla	Low fat, vanilla
Milk, 2% fat	0	0	66.09
Skim milk	70.00	71.01	0
Water	9.88	10.55	18.10
Sucrose	12.79	12.28	10.10
Starch	4.41	5.00	4.10
Sodium stearoyl lactylate	0.20	0.20	0.20
Sodium alginate	0.15	0.10	0.23
Flavor and colors	0	0.70	0.36
Polyphosphates (50% tetrasodium and 50% sodium acid pyrophosphate)	0	0.08	0
Cocoa	2.57	0	0

Adapted from: King and Leshik, 1993: Leshik, 1993

Low-fat and Nonfat Puddings

Low-fat and nonfat pudding products offer consumers choices for nonfat or low fat versions of puddings. In the absence of fat in the mix, calcium-sensitive, irreversible gelling hydrocolloid, namely sodium alginate, is a key ingredient to form the desirable texture in UHT-processed pudding. Low methoxy pectin and gellan gum are also effective (King and Leshik, 1993). Table 17.22 gives the formulation of nonfat and low-fat pudding. The processing procedure is similar to that of other sterilized, aseptically packaged products.

No-Sugar-Added and High-Protein, Low-Carbohydrate Pudding

The formulation of no-sugar-added pudding is exemplified in Table 17.23. This formulation uses lower milk solids to obtain stability at pH of 5.7, and xanthan gum functions as a viscosity and body-building agent. The key to formulating this type of pudding is the use of high-intensity sweeteners, namely aspartame, sucralose, and acesulfame-K. It is common to use a mixture of the high-intensity sweeteners to impart a better sweetness profile resembling sucrose. The process involves two stream steps. In the first step, an aqueous solution of aspartame containing all of the lactic acid is filter-sterilized and stored aseptically. The second stream consists of the rest of the formulation ingredients which are blended and homogenized at 57°C (135°F) in a two-stage homogenizer (14 MPa [2,000 psi], first stage; 3.5 MPa [500 psi], second stage). The mix is then sterilized by heat treatment of 130°C to 150°C (266°F to 300°F) for 6 to 30 seconds (Leshik et al, 1990). The two sterile streams are then blended aseptically at 24°C (75°F); thus, heat-labile aspartame is not subjected to the high temperatures required for sterilization. The sterilized pudding is then aseptically packaged. In the final pudding, the lactic acid acidifies the pudding to reduce the pH from 6.6 to 5.6 and the pudding obtained does not exhibit an acidic taste.

Table 17.23. Formulation of no-sugar-added pudding.

Ingredient	%
Milk, 2% fat	62.63
Modified starch	4.30
Flavor	0.21
Sodium stearoyl lactylate	0.20
Coca	2.80
Water	29.26
Lactic acid	0.31
Aspartame	0.14
Xanthan gum	0.15

Adapted from Leshik et al. (1990)

The high protein, low carbohydrate pudding formulation is shown in Table 17.24.

Table 17.24. Formulation for high-protein, low-carbohydrate pudding.

% Composition	Vanilla	Chocolate	Butterscotch	Banana
Water	84.7	83.78	80–90	80–90
Calcium/sodium caseinate	7.46	6.87	6–9	6–9
Soy protein isolate	5.56	5.56	4.5–7.5	4.5–7.5
Whey protein isolate	0	0	0–3	0–3
Whey protein concentrate	0	0	0–1.5	0–1.5
Soybean oil	0.88	0.58	0.75–1.75	0.75–1.75
Sodium chloride	0.44	0.44	0.1–0.5	0.1–0.5
Potassium chloride	0		0–0.4	0–0.4
Carrageenan	0.3	0.33	0.1–0.35	0.1–0.35
Dipotassium phosphate	0.36	0.12	0.3–0.45	0.3–0.45
Tricalcium phosphate	0.044	0.044	0.025–0.3	0.025–0.3
Sucralose	0.04	0.04	0.03–0.05	0.03–0.05
Acesulfame-K	0.033	0.033	0.03–0.04	0.03–0.04
Vanilla flavor	0.19	0	0	0
Cocoa	0	2.21	0	0
Butterscotch flavor	0	0	0.1–0.05	0
Banana flavor	0	0	0	0.1–0.5

Adapted from Scinto (2006)

Table 17.25. Typical formulation of parfait-type (layered) pudding.

% Composition	Layer 1: vanilla	Layer 2: chocolate	Layer 3: vanilla
Water	48.87	49.5	48.13
Condensed skim milk	22.64	22.0	24.03
Sugar	17.17	17.0	17.17
Hydrogenated coconut/palm kernel oil	5.62	4.6	5.62
Modified food starch	3.8	3.8	3.8
Whey protein concentrate, 35% protein	1.00	0	0
Phosphoric acid solution, 17.5%	0	0	0.32
Flavor	0.55	0.2	0.55
Sodium stearoyl lactylate	0.2	0.2	0.2
Salt	0.18	0.2	0.18
Cocoa powder	0	2.5	0

Adapted from Flango et al. (1990)

Layered Pudding for Parfait Configuration

Multilayered desserts made with pudding (Table 17.25) may be decorated with a layer of whipped cream or fruit. After processing each layer of pudding, transparent cups are partially filled with layers of vanilla, chocolate, and vanilla puddings. Each layer should be distinctly visible with a clear-cut demarcation between the layers. Aseptic pudding with puree has been described by Welch (2007).

Flan Type Pudding

Flans are moldable gels that can be easily removed from their containers by placing the container upside down and punching a hole on the bottom. Flan may have a caramel sauce topping at the bottom of the container which flows from the top of the product after it is removed. The texture may be firm with brittle, creamy, or cohesive mouth feel. Eggs and the baking procedure develop the desirable firm texture of egg custard. The manufacturing procedure for flan is covered in a U.S. patent (Salmones, 2006). Table 17.26 shows a formulation of baked pudding, and Table 17.27 is shows the formulation of an egg-free flan.

In the batch skim milk is pumped into the processing vat, followed by the addition of cinnamon, lemon rind, cream cheese, liquid

Table 17.26. Typical formulation of baked flan.

Ingredient	Usage level
Skim milk	65 liters
Cream cheese	15 kg
Liquid eggs	20 kg
Sugar	14 kg
Flavor, cinnamon	125 g
Lemon rind	550 g
Liquid caramel	6 kg

Adapted from Salmones (2006)

Table 17.27. Formulation of egg-free flan.

Ingredient	%
Water	81.670
Nonfat dry milk	7.350
Sugar	8.160
Rice flour	2.000
Carrageenan	0.282
Locust bean gum	0.116
Pectin	0.065
Tetra potassium pyrophosphate	0.212
Xanthan gum	0.073
Whey protein isolate	0.073

Adapted from Kadan and Ziegler, Jr. (1990)

eggs, and sugar. The mix is blended for 30 minutes. Liquid caramel (5 to 6 g) is placed into 120-ml containers, followed by 100 to 105 g of the blended mix in each packaging cup. The containers are vacuum sealed and heat treated at 70°C to 105°C (158°F to 221°F) for 25 to 35 minutes. The set flan is cooled rapidly to 0°C to 8°C (32°F to 46°F). Flan also can be made without caramel topping or with other flavors. For coffee flavor 2% to 3% soluble coffee is used, and for chocolate, a 10% to 12% dark chocolate coating is used.

Aerated Dessert/Mousse

Mousse is an aerated and stabilized product with a light, dry, and foam-like consistency. Generally, mousse is prepared from a heat-treated liquid mix that is whipped in a continuous aerator, dispensed into cups, sealed, and cooled. Mousse also can be produced with an ice cream freezer, followed by packaging of frozen mousse in cups. The frozen mousse is marketed frozen and is thawed prior to consumption at home by the consumer. The density of the product is controlled by the degree of whipping air and the resulting overrun. The overrun may be 100% to 150%, depending on the texture desired in the whipped product. It is important to use an effective emulsifier for the manufacture of mousse to create fine air bubbles. Gelatin is a key ingredient along with cream, cream cheese, and sugar. Flavorings such as fruits, cocoa, or vanilla may be used. Flynn (1999) has given the formulation (Table 17.28) and process of such a product.

To manufacture chocolate dessert, cream, sugar, cocoa, and chocolate chips are blended and beaten in a Hobart-type mixer to attain 25% overrun at 10°C. This pre-mix is stored

Table 17.28. Suggested formulation of settable and aerated dessert.

Ingredient	Chocolate	Lemon	Strawberry	Vanilla
Cream, 35% fat	16 liters	10 liters	10 liters	10 liters
Sugar	3 kg	2050 g	2.5 kg	2050 g
Gelatin	226 g	226 g	226.0 g	226 g
Cocoa, alkalized, 10%–12% fat	600 g	0	0	0
Chopped chocolate	3 kg	0	0	0
Cream cheese	1,200 g	1,240 g	2 kg	1,250 g
Lemon juice	0	2.7 liters	0	0
Condensed milk	0	4 kg	0	4 kg
Water	1,200 ml	2 liters	2 liters	2 liters
Strawberries	0	0	1 kg	0
Vanilla	0	0	0	As needed

Adapted from Flynn (1999)

frozen or refrigerated for use in the final product. Five liters of pre-mix is whipped, mixed with 1.5 gallons of cream, and whipped further. It is then mixed with a slurry consisting of 300 ml of water at 80°C, 300 g cream cheese, and 55 g of gelatin. The product is reported to have a shelf life of six months in frozen storage, and it exhibits a shelf life of three weeks in refrigerated conditions (Flynn, 1999).

To manufacture lemon mousse, cream cheese and sugar are mixed in a Hobart mixer to the consistency of a paste. The paste is whipped to obtain 25% overrun at 10°C (50°F) and the cream is blended and whipped further. At this point, lemon juice is added. Gelatin is dissolved in hot water and blended in. After mixing, the filling is ready for parfait rolls, cheese cakes, or Swiss rolls.

For strawberry mousse manufacture, sugar, cream cheese, and cream are blended to a smooth consistency and whipped to obtain 25% overrun at 10°C (50°F). Strawberries are then blended into the mix. The gelatin is dissolved in hot water (80°C, 176°F) and added to the aerated blend while continuing the whipping process for 10 to 15 seconds. The mousse may be stored frozen or refrigerated.

The vanilla product is made by blending cream cheese and sugar in a Hobart bowl, followed by the addition of condensed milk and cream. The mix is whipped to 25% overrun at 10°C (50°F). The gelatin solution obtained by dissolving gelatin in hot water (80°C; 176°F) is then blended along with vanilla flavor for 10 to 15 seconds. The mousse is ready for packaging and storage under frozen or refrigerated conditions.

Pudding with Particulates

Puddings with particulates such as rice and tapioca require sterilizing conditions and processes that are distinctly different from those used in smooth, single-phase puddings in which starches *per se* are used.

Table 17.29. Suggested formulation of aseptically processed rice pudding.

Ingredient	%
Water	62.7
Heavy cream	12.0
Nonfat dry milk	5.4
Sugar	11.2
Rice	7.0
Flavor/color	0.6
Carrageenan	0.5
Egg yolk	0.34
Sodium stearoyl lactylate	0.2
Trisodium pyrophosphate	0.04
Sodium acid pyrophosphate	0.02

Adapted from Lo et al. (2000), Budinoff (2003)

Selection of rice. Rice-based dairy desserts may be prepared from whole or broken rice and grain fractions. Rice pudding containing whole rice grains is popular. In the production of rice pudding, the cooking parameters are critical for the product quality. In general, rice containing high amylopectin content is avoided because amylopectin fraction of starch leads to retrogradation and the rice texture becomes too firm during refrigerated storage of the pudding. Table 17.29 shows the composition of aseptically processed rice pudding.

Cooking characteristics of rice. The amount of water needed to cook rice is an indication of the approximate increase in size of the grain (swell). However, rice grain increases more than twice in volume, even if it is cooked in only twice its volume of water. Long grain rice tends to swell more than short grain rice, and parboiled rice swells less than non-parboiled rice. Long grain rice gives more intact kernels in rice pudding than short grain rice. Short grain rice in ultrahigh heat processing becomes less firm than long grain rice, but may develop an excessively mealy and grainy texture during refrigerated storage. Brown rice or unpolished rice swells somewhat less than polished rice. Rice swells more when cooked in milk than in water. The texture of the cooked rice is an important determinant of its acceptability.

Batch process procedure. In a typical procedure, rice pudding is prepared by immersion of pre-soaked rice (5% to 6% by weight of milk) in simmering milk followed by addition of sugar (6% to 8%) and heating the mixture further until the rice softens and shows signs of gelatinization, leading to substantial thickening. Rice grains (broken or whole) are exposed to saturated steam at a pressure higher than atmospheric pressure (greater than 0.03 bar) in an autoclave for sufficient time to gelatinize a major portion of the starch. The rice grains are then mixed with milk.

The production process for in-can sterilized creamed-rice dessert involves short grain rice releasing starch on cooking (Aneja et al. 2002). Homogenization of milk improves the product quality. Gradual heating periods and agitation are necessary during autoclaving to prevent localized overheating. The product exhibits some browning and age thickening during its shelf life of 12 months. Long grain or medium grain rice, milk, sugar, eggs, vanilla, and salt are added to a batch cooker. Heat processing involves a temperature of 88°C (190°F) and holding period of 30 to 40 minutes, with gentle stirring. The product is hot-packaged in a clean environment, vacuum sealed, and cooled to 4°C to 5°C (39°F to 41°F). The extended-shelf-life product is marketed refrigerated and has a shelf life of 60 to 75 days.

UHT process procedure. Rice pudding also has been manufactured employing a scraped-surface heat exchanger. Rice and milk are pumped into the scraped-surface heat exchanger system, heated to 115°C (239°F), held for 26 minutes in a holding tube, and then cooled to 80°C (176°F) in another cylinder. The rice pudding is transferred to a sterile buffer tank, and then dispensed into form-and-fill plastic containers under aseptic conditions. This process provides a better shelf life by subjecting the product to a more rigorous heating regime that kills the microbial vegetative cells as well as their spores. The product is refrigerated to retain freshness.

A flow chart for manufacturing long-life rice pudding is given in Figure 17.10.

Quality Control Essentials

During the manufacturing processes, it is imperative to ensure optimal cooking of the starch by appropriate heating of the pudding mix. If the starch is undercooked, its appearance is cloudy, its texture is thin, and the viscosity is too low. On the other hand, overcooked starch loses viscosity and acquires cohesive long texture. Optimally cooked starch is clear and maintains stability with good viscosity and heavy-bodied short texture.

The processing conditions should be optimized depending on the UHT equipment being used. Preheating is not required in direct steam heating systems and in scraped-surface heat exchangers, whereas for plate heat exchangers preheating at 65°C to 76°C (149°F to 169°F) (no holding period) is recommended. Homogenization is not recommended for direct steam heating equipment. For plate heat exchanger equipment, homogenization at 20 kg/cm^2 helps to avoid sandiness in the product. Similarly, homogenization at 75 kg/cm^2 for scraped-surface heat exchangers is given (Rapille and Vanhemelrijck, 1998). According to the authors, effective heat treatments are also specific for the type of processing equipment. In general, the optimum heat treatment for direct steam heating equipment is 142°C (288°F) for 5 seconds, a plate heat exchanger requires 140°C (284°F) for 10 seconds, and scraped-surface heat exchanger equipment needs 138°C (280°F) for 5 seconds.

Sensory evaluation should be performed on fresh and stored samples for appearance, flavor, and texture. A score sheet should be developed and the terms used to describe the attributes should be fully understood by the sensory panel. Any free liquid on

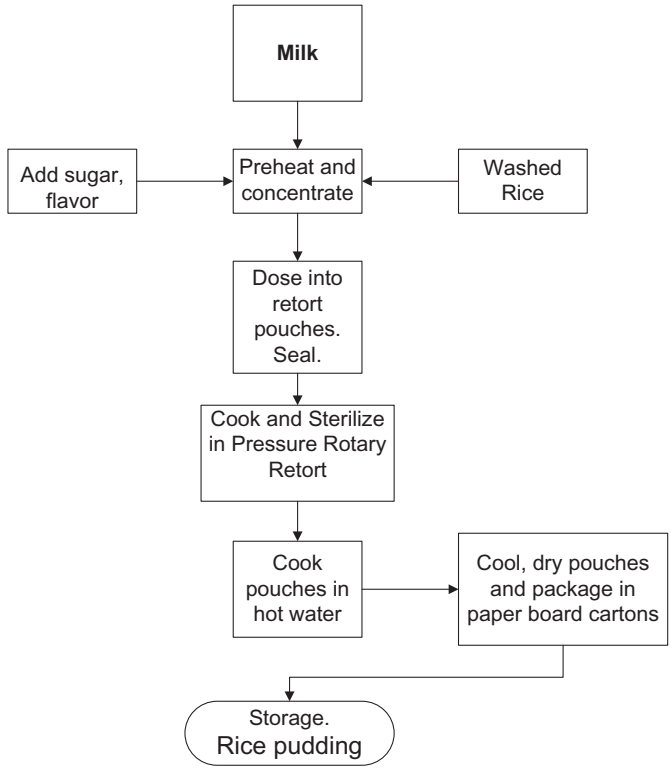

Figure 17.10. Flow chart for manufacturing long-life rice pudding.

the surface or sides of the product should be noted.

Viscosity, hardness, and cohesion values for dairy desserts have been suggested (Rapille and Vanhemelrijck, 1998). Viscosity measurements were made at 20°C (68°F) with a Haake Rotoviscometer type RV2, using the coaxial measuring unit MV1. Viscosity is expressed as MPas as calculated from the shear stress at a shear rate of 25 min^{-1}. The textural values (hardness and cohesion) were assayed on a Instron Universal Testing machine, model 1140, using a compressor load cell type CBM 50 N and applying the general texture profile method. The authors recommend that for creamy smooth pudding, the viscosity (mPas) should be 750 to 1,000, hardness (N) should be 0.5 to 1.0, and cohesion should be 0.7 to 0.9. For gelled-type desserts, the recommended viscosity, hardness and cohesion values should be 650 to 1,000, 1.0 to 1.5, and 0.5 to 0.7, respectively.

Microscopic examination (under polarized light) by counting the number of non-gelatinized starch particles in the heat-processed pudding mix should reveal the degree of the starch gelatinization. The degree of starch degradation can be evaluated under microscope by observing the nature of swollen starch granules.

The overrun is a measure of the volume of air or gas whipped in the aerated product. The percent overrun in yogurt mousse is 50% to 80%, whereas in whipped dairy mousses, it ranges from 100% to 130%. For whipped creams, it is in the range of 200% to 250%. Because overrun has a profound effect on the texture and mouth feel of whipped products, it is essential to control the overrun in the product.

Shelf life studies of the products stored at 4° to 5°C (39.2°F to 41°F) as well as under abuse conditions should be monitored systematically. The samples should be evaluated for flavor and textural degradation as indicated by deleterious changes in viscosity, shortness, gel strength, lumpiness, creaminess and syneresis.

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Chapter 18

Dairy Ingredients in Bakery, Snacks, Sauces, Dressings, Processed Meats, and Functional Foods

Ramesh C. Chandan

Dairy ingredients are commonly used in the manufacture of various food items. As shown in Figure 18.1, an array of dairy ingredients is derived from milk and colostrum by various unit processes which involve concentration, dehydration, crystallization, membrane fractionation of milk constituents, and physical isolation of physiologically active nutrients. Lactose is used as an extender in pharmaceutical industry and its derivatives lactitol, lactulose, and lactobionic acid are useful pharmaceutical compounds. Lactic acid polymers are used in biodegradable plastics and packaging materials.

Several bioactive peptides derived from enzyme hydrolysis of fractionated casein and whey proteins have been developed as functional food ingredients. Functional bioingredients from dairy fermentations are discussed in Chapter 14, and Nutritive and health characteristics of dairy ingredients are given in Chapter 16.

Dairy-derived ingredients have distinct functionality and nutritive profiles suitable for use in bakery foods, infant formula, nutritional and dietetic drinks and bars, chocolate and confectionery products, snacks, sauces, dressings, and processed meats. Furthermore, certain biopeptides form the basis of nutraceuticals with purported health benefits including sports performance enhancement, blood pressure control, anti-anxiety effects, and sleep-inducing ability.

The applications of dairy ingredients in diverse foods are discussed in various chapters of this book. The use of dairy ingredients in dairy foods is given in Chapter 17; in chocolate and confectionery products in Chapter 19; and in infant formulas, nutritional beverages, meals, and bars in Chapter 20. This chapter includes applications in functional foods, bakery, snacks, sauces, dressings, and processed meats.

Bakery Products

Bakery items acquire desirable attributes of soft crumb, flavor, and crust color superiority when nonfat dry milk (NFDM), milk fat, whey solids, whey protein concentrates, and sodium/calcium caseinates are included in their formulation. These ingredients provide functional properties as well as desirable nutritional superiority. In particular, the amino acid profile of dairy proteins complements and develops the improved amino acid profile of grain proteins. Economical substitute blends with functional attributes comparable to NFDM have been developed. Dry sweet whey as such is widely used in bakery items, especially cookies, because of its superior functionality with respect to color and texture formation in the finished product.

Ingredients used in baking primarily consist of flours made from hard and soft wheat, miscellaneous flours of other grains, sugars and syrups, shortening, milk-based

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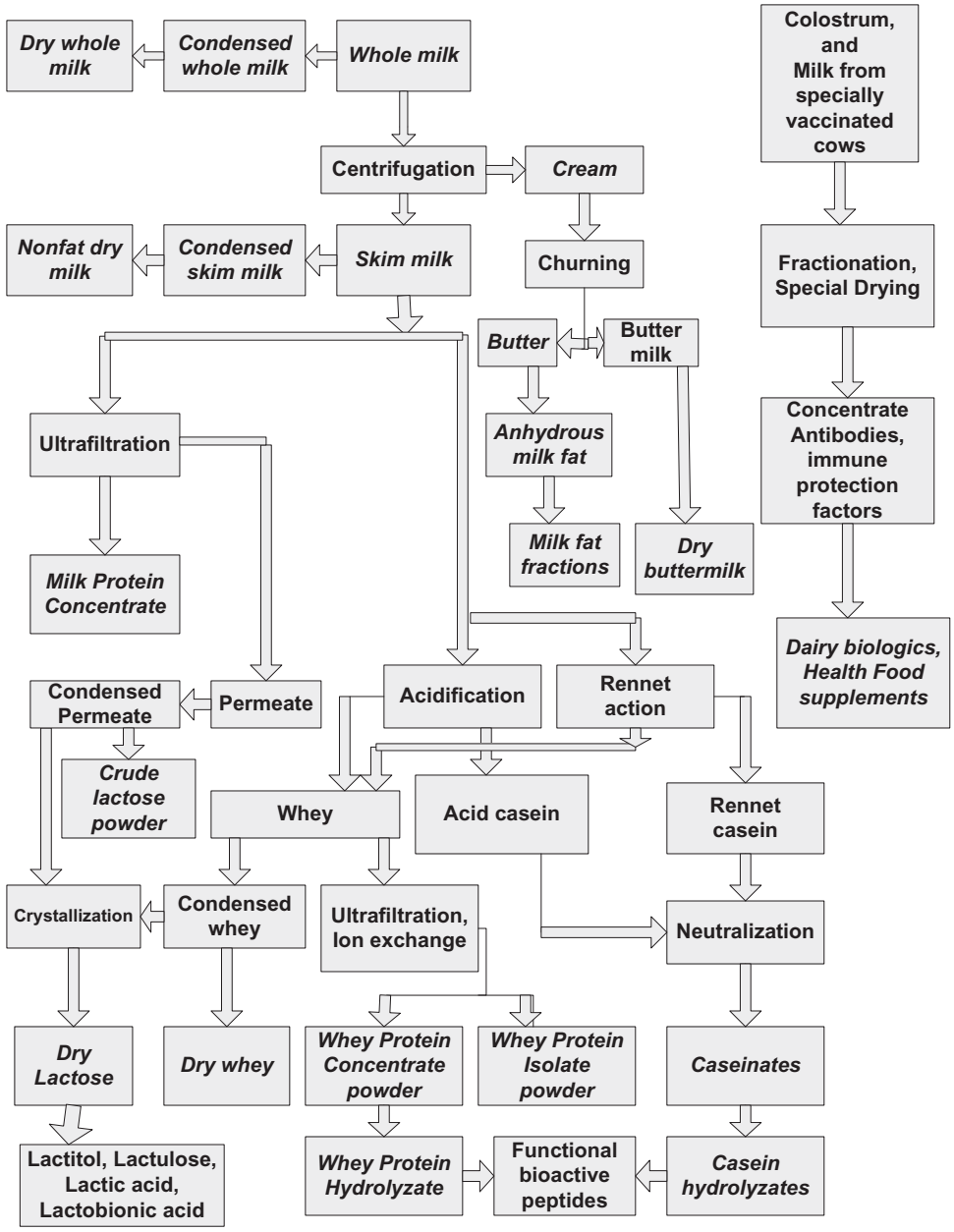

Figure 18.1. Major dairy ingredients and their origin. Adapted from De Wit (2003)

ingredients, eggs and egg products, and water. Flours, sugars, water, and shortenings are major and essential components of most bakery items. Bakery items that benefit from dairy ingredients include cakes, crusty breads, rolls, bagels, cookies/biscuits, pies, Danishes, muffins, croissants, and puff pastries. Dairy-derived ingredients assist by functional attributes of emulsification, gelation, aeration, browning, and flavor develop-

ment. They are necessary for desirable flavor, color, texture, and structure of the bakery product. Presently there are market trends to offer low-fat, high-protein, and low-carbohydrate foods. Furthermore, there is a strong interest in functional foods and products with no trans-fatty acids (TFA). Dairy-derived ingredients offer interesting tools to develop the trendy bakery products.

Dairy Ingredients in Baked Foods

Liquid Milk

Ideally, whole milk or its concentrates yield baked goods of better flavor, which may be considered premium products. However, fresh milk has a relatively short shelf life and bakeries are not equipped to handle this ingredient. The excessive moisture of liquid milk also is a drawback for most bakery formulations. Accordingly, fresh milk is not normally directly used in most bakeries. In reality, byproducts from the dairy industry primarily are used to satisfy economic conditions of commercial bakery business. Milk solids from nonfat dry milk, dry whey, dry buttermilk, and condensed milk constitute the ingredients of choice. The dry dairy ingredients provide convenience of use, long shelf life, and savings in transportation and storage costs. Butter, anhydrous milk fat, and cheese also contribute unique flavor and functional attributes.

Nonfat Dry Milk

Milk sugar and milk protein are functional milk constituents (other than milk fat) of interest to bakers. Dry whey and crude lactose products constitute an economical source of lactose, whereas nonfat dry milk, dry buttermilk, and dry whey protein concentrates provide lactose and milk protein functionality to baked goods. Sodium and calcium caseinates and whey protein isolates are sometimes used for their protein's distinct functional properties. Whole milk powder can replace nonfat dry milk and butter. Various bakery blends derived from dairy ingredients, defatted soy flour, emulsifiers, and dough conditioning agents are available to the baker for customized use. Chapter 6 of this book discusses dry milk and dry milk products in detail.

Dried Whole Milk Powder

When the formulation of a bakery product requires milk or NFDM and butter, it is advantageous to use a single ingredient, dried whole milk (DWM), which has a shelf life of six to nine months under ambient storage conditions. It functions similarly to NFDM in that it enhances water absorption attributes in bakery items, resulting in desirable color, texture, and structure. Nutrients contributed to bakery foods include the nutrient contribution of NFDM. However, the milk fat content of DWM also gives a dairy flavor in addition to supplying the fat-soluble vitamins A, D, E, and K, and essential fatty acids associated with milk fat. Furthermore, whole milk powder retards staling of bread.

Whey and Whey Products

Whey and whey products have been used in many bakery applications (Stollar, 2009). Information on whey and whey products is provided in Chapter 8. Dry whey is used as a replacement for NFDM in bakery formulations at 2% to 4% of the flour weight. Dry whey is high in lactose, which accounts for most of the color formation (Millard reaction) in crust. In addition, caramelization of lactose during baking gives a characteristic aroma and flavor.

Whey protein concentrate (WPC) containing 34% protein simulates NFDM in composition, but due to its whey protein content in entirety, it possesses superior nutritional value compared to NFDM. Whey protein products have good emulsifying quality in high-fat bakery items because they contain

both hydrophobic and hydrophilic groups. Consequently, they help to distribute fat in the dough, with the result that the baked product has good porous structure and volume. Whey proteins denature at baking temperatures; thus, they form a heat-induced gel with good water-binding ability. This effect contributes to moistness, tenderness, and enhanced keeping quality of bread, rolls, and buns.

WPC assists in improving the machinability of dough. WPC with 50% to 80% protein content as well as whey protein isolate (more than 90% protein) are also available with low lactose levels for specialty use to satisfy requirements for low-carbohydrate, low-glycemic, high-protein, and reduced-fat bread products (Burrington, 2005). Also, by using WPC, it is possible to reduce about 25% of the carbohydrates of bakery goods while improving bread flavor, crust browning, toasting quality, and crumb structure, and slowing down the staling process (starch retrogradation).

WPC products assist in whipping and foam formation in angel food cakes and meringues. In yellow and sponge cakes, a fraction of egg albumin can be substituted by WPC. In pastries and biscuits, use of WPC in prebake glazes produces desirable color and gloss. Including WPC in the formula helps to disperse shortening and enhance its functionality. The water requirements of bakery formulation must be assessed and adjusted because water absorption characteristics increase as the whey proteins undergo heat denaturation. Due to the high lactose content, the use of dry whey requires adjustment in process time and temperature of baking to maintain yeast growth and CO_2 production.

The nutrient profile of whey ingredients complements the nutritional value of baked goods. Whey protein is recognized for its high content of branched-chain amino acids (26 mg of leucine, isoleucine, and valine/100 g of WPC). They provide 10% to 15% of the total energy required during endurance exercise. Furthermore, on digestion, WPC produces bioactive peptides which have been documented to reduce blood pressure, modulate immunity, and display activity against viruses and other infections (Chapter 16).

Lactose

Food grade and crystalline lactose (greater than 99.5% lactose) is commonly used in infant formula manufacture. In addition, dry permeate powder products obtained from ultrafiltration of skim milk and whey are predominantly lactose products. These cost-saving products are available for use in bakery items. Lactose functions include relatively low sweetness and enhanced retention of moisture for crumb improvement. Lactose shortens proofing time, and increases volume and gas retention. The dough rises faster and color is achieved quicker at even lower temperatures of baking. Lactose extends shortening, making fat reduction possible. It produces darker crust color and softer crumb in yellow cakes and sponges.

From a nutrition standpoint, lactose has a relatively low glycemic index of 46, as compared to 100 for glucose and 60 for sucrose. However, individuals with lactose maldigestion condition must exercise caution not to exceed their threshold of lactose dose to avoid the unpleasant symptoms associated with lactose intolerance.

Butter and Anhydrous Milk Fat

Fats are selected for their use in formulating baked goods based on their performance during manufacture of the bakery items and the subsequent consumer attributes that are imparted. Real and perceived health concerns also dictate selection of fat use in bakery items. Reduced use of saturated fat and increased use of unsaturated fat has prompted the use of certain fats with lower saturated fatty acid composition. The trend also

includes avoiding the use of hydrogenated fats in all foods.

Two types of butter are used in bakeries worldwide. Sweet cream butter is manufactured from cream by churning (Chapter 9) and is available in salted or unsalted forms. Another type of butter is obtained by churning of cream cultured with *Lactococcus lactis* subsp. *lactis*. Other subspecies of the culturing organism are *cremoris* and *diacetylactis*. In addition, the Leuconostoc species may be used for the generation a potent flavor compound (diacetyl). The cultured butter is more prevalent in Europe, and sweet cream butter is generally used in North America and the United Kingdom.

In general, several functional properties of fats, including butter in baked products, are significant (Chandan, 1997). Fats cause tenderization by preventing formation of a three-dimensional starch-protein network. They exert a shortening effect or texture modification of dough and baked goods. Fats also assist in aeration during mixing or creaming quality, and exert an anti-staling effect and shelf life extension by slowing retrogradation of starch. They modify the consistency of dough, help in heat transfer, and act as carriers of flavors. They create flakiness in laminated bakery items. Fats also interact with gluten in yeast-raised dough and with starch, leading to modification of hydration potential. Finally, they assist in release from pans. Butter and butterfat products provide exceptional functionality, as discussed below.

Flavor. The flavor of butter is characteristic and an asset for the baked product. In addition to lending to the upscale or gourmet image, butter imparts a distinctive flavor to biscuits, breads, frostings, icings, butter creams, cookies, crackers, croissants, muffins, cakes, pastries, and pie crusts. It is a good carrier of flavor through the bulk of the product. Reaction flavor compounds, e.g. lactones, are generated from fatty acids of butterfat during baking. To meet consumer demand for lower calorie and reduced-fat products, enzyme-modified butterfat products can be used to give intense flavor notes of butter at low usage levels.

Butter assists in even distribution of added flavorings throughout the dough and the product. Lecithin, naturally present in butter at the 0.25% level, is instrumental in stabilizing fat emulsion, leading to consistency of texture, aroma, and flavor in baked foods. In this regard, butter and dry buttermilk perform better than anhydrous milk fat, which contains essentially no lecithin.

Mouth feel. Another functional attribute of butter is related to body and texture of bakery items. Butter imparts highly desirable mouth feel attributes to the baked products. This property is related to the ratio of crystalline (or solid fraction) and non-crystalline or (liquid fraction) of butter as a function of temperature. Solids fat index (SFI) is related to percent solid fat content of a fat (Chandan, 1997). Bakery fats have different SFIs as shown in Table 18.1.

Butter melts completely at 37.8°C (100°F). At body temperature only 5% is in solid form, thereby giving no waxy mouth feel. Butter exhibits a steep and sharp SFI curve along with a low melting point range of 27.8°C to 37.2°C (82°F to 99°F). It is commonly combined with some margarine and shortening products to alleviate the waxy mouth feel observed when only relatively broad SFI-curve shortenings (e.g., puff pastry margarine) are used.

Flakiness. Butter serves a useful function in imparting flakiness in croissants, Danishes, and puff pastry. Butterfat is applied in between dough layers, and the thin layers of milk fat between the dough layers disrupts the gluten from forming a three-dimensional network. The butterfat layer is enhanced further by rolling and folding process. Upon baking, the dough layers rise due to steam formation from the moisture in the butter, followed by release of carbon dioxide from leavening. In pie and tart doughs, an increase

Table 18.1. Solid fat indices of bakery fats.

Fat source	% solids content at:				
	10°C (50°F)	21.2°C (70°F)	26.7°C (80°F)	33.3°C (92°F)	37.8°C (100°F)
Butter	32	12	9	3	0
Lard	25	20	12	4	2
Table margarine	28	16	12	3	0
Roll-in margarine	25	21	20	18	15
Puff margarine	28	25	24	22	19
All purpose shortening	33	28	22	10	8
Cake and ice shortening	28	23	22	18	15

Adapted from Chandan (1997)

in flakiness is accomplished by partial mixing of butter in the dough and entrapped carbon dioxide.

Shelf life. Like other shortenings, milk fat from butter, anhydrous milk fat/butter oil provides a barrier to loss of moisture and air following the baking process. Furthermore, retrogradation of starch (associated with staling) is inhibited. Thus, tenderness and flakiness are maintained during the shelf life of baked goods including cakes and yeast- or chemically leavened breads.

The shortening attributes of butter are related to its ability to disrupt the development of the gluten network, thereby impacting the texture of bakery products. Milk fat coats individual particles of flour, which causes shortening of the gluten network. In this regard, milk fat is solid enough (fine crystal structure) to prevent absorption by flour but is liquid enough to form a coating on flour particles, and thus imparting better shortening properties in cakes and cookies. In cake batters, milk fat is creamed in the presence of sugar to assist in adequate air incorporation, which is related to the size and number of crystals. Batter with good aeration development leads to large numbers of small bubbles, which, on baking give cake its characteristic structure. Butter held at 18°C to 21°C (64°F to 70°F) is quite workable.

To fulfill a need for enhanced functionality in milk fat, the dairy industry has developed fractionated and plasticized butterfat for specific use in pastry cookie and cake manufacture. Milk fat consists of three major melting fractions: Low-melting glycerides melt below 10°C (50°F), middle-melting glycerides melt in the range of 10°C to 20°C (50°F to 68°F), and high-melting glycerides melt above 20°C (68°F). Low-melting glyceride (LMG) fraction prevents the appearance of fat crystals during the shelf life of shortbread cookies made with butter (fat bloom defect). LMG fraction also traps a higher concentration of lactone and methyl lactone flavor precursors. Therefore, its use assists in developing a more intense butter flavor in the product. Butter made from anhydrous milk fat enriched with high melting glycerides duplicates functionality of nondairy pastry fat in croissants and Danish pastries. The ingredient cost is considerably moderated by blending vegetable oil with butterfat in a near 1:1 ratio. Butter in powdered form is also available for use in dry bakery mixes.

The aeration capability of butter in a cake formula is enhanced by the addition of 1% monoglyceride (∝-form) based on batter weight (DMI, 2006a).

Handling of Butter in Bakeries

Storing butter in its original cartons protects it from acquiring foreign flavors and aromas. It should be stored away from foods with strong odors. Storage at temperatures of 0° to 3°C (32°F to 38°F) and 80% to 85% relative humidity for a maximum of four months

is advisable. Butter can be held for a year in frozen form −23°C to −29°C (−10°F to −20°F).

Butter should be tempered to the appropriate temperature for optimal functionality. Softening of butter is advisable at 15.6°C to 18.3°C (60°F to 65°F). The recommended thawing times are two to three days for 30-pound cases and four to five days for 68-pound blocks. Accelerating the thaw time by subjecting butter to higher temperatures interferes with the crystal profile and should be avoided. Liquefied warm butter gives a greasy dough with undesirable viscosity or density, and may compromise the quality of the baked product. Cold butter works well in pie and pastry dough production. Prior to the addition of flour, sugar and other ingredients, cold butter (to be used as a roll-in fat) is made pliable by beating it with a hook or paddle in a Hobart mixer. In the production of cookies, cakes, breads, and icings, butter is warmed to room temperature. For cake batter production, sugar is thoroughly creamed with butter at room temperature to achieve uniform distribution into the batter or dough. Antioxidants (BHA and BHT) at the 0.005% level may be added to long-life baked foods (for instance, cookies) to avoid oxidative deterioration of the fat.

Cheese and Cheese Products

Cheese imparts a distinct flavor to bakery products. Colby imparts a mild, smooth body, while parmesan gives a sharp flavor (Chapters 10 and 11). Other cheeses used in baking include asiago, cheddar, Monterrey jack, mozzarella, and Swiss. Aged cheeses are used to create cheese flavor even at low-cheese levels in bakery goods. Enzyme-modified cheeses contribute several times more potent flavor than their regular cheese counterparts. The protein and lactose of cheese gives surface browning to bakery items. In addition, cheese modifies the texture depending on whether it is very hard, hard, semi-soft, or soft. High-moisture and high-fat varieties impart a smooth mouth feel. Cheese fat melts at 28°C to 36°C (82.4°F to 98.6°F), which is synonymous with body temperature. Accordingly, complete melting of milk fat contributes to a smooth mouth feel; for instance, cream cheese is noted for a contributing smooth texture to bakery products. Other cheeses such as asiago and parmesan contribute their dense or crumbly texture.

The process cheese category is especially suitable for bakery applications (Chapter 11). Restricted-melt cheeses offer low flowability without loss of identity; thus, they are suited for breads, extruded fillings, and pretzels. A cheese slurry consisting of cheese, enzyme-modified cheese, and cheese flavors can be incorporated in dough and baked to yield baked cheese crackers. Cheese powders, which come in a variety of flavors and can be blended in bakery dough, offer the advantage of shelf stability. Popular cheese flavors are cheddar, blue, colby, Monterrey jack, mozzarella, Romano, and parmesan. Cheese powders are convenient to use as a seasoning for covering the surface of potato chips and extruded grain snacks.

Because cheese is a concentrated form of milk protein, fat, minerals, and fat-soluble and water-soluble vitamins, its incorporation contributes its share of the overall nutrient profile to bakery items. These nutrients include high-quality milk protein, calcium, riboflavin, and vitamins B_{12}, A, D, E, and K. Furthermore, cheese is low in undesirable trans-fatty acids and it furnishes desirable essential fatty acids (linoleic and linolenic acids).

Pizza

Pizza topping constitutes a major application of cheeses. The most commonly used cheeses for pizza are mozzarella, provolone, parmesan, and Romano. Mozzarella is the dominant topping cheese; the low-moisture variety is preferred. Regular moisture whole milk

mozzarella is seldom used on pizza. Low-moisture mozzarella may be made from whole milk or part-skim milk. The part-skim low-moisture mozzarella has an advantage over the whole milk variety in that it does not oil-off as a topping, possesses a longer shelf life, and is easier to process in a pizzeria. It is commonly used in shredded, chopped, or diced form. Fresh mozzarella is another variation; it comes in small balls packed in water. Fresh mozzarella is sometimes referred to as scarmorze or scamorze. Because of its high moisture content, fresh mozzarella has a short shelf life of 10 days. Nevertheless, some pizza makers use it on their pizza.

The age of cheese affects its flavor, texture, and performance in pizza. Mozzarella should be aged for 13 to 20 days under refrigerated storage to obtain a yellowish color, more defined flavor, and softer texture. If the cheese is not ripened properly, it is white in color and bland in flavor, and possesses a hard, rubbery consistency. On pizza, it does not melt properly, does not stretch well, and does not brown satisfactorily on baking. Over-ripened mozzarella is too runny, loses stretch, and may display more oil separation on baking.

Some gourmet pizzas also contain asiago, fontina, caciocavallo, Bel Paese, teleggio, gorgonzola, and ricotta cheeses. In addition, for flavor and texture variations, there is a trend to use non-Italian cheeses such as cheddar, brick, Monterrey jack, Muenster, feta, gouda, colby, and Swiss or gruyere, which may be blended with mozzarella cheese. Cheese applications in various foods are enumerated in Chapter 11.

Dairy Management Inc. has publishes a series of application manuals to demonstrate the uses of butter (DMI, 1996a), whey products (DMI, 1996b), cheeses (DMI, 1997), lactose (DMI, 1998), and concentrated and dry milks (DMI, 2003). Some key applications of dairy ingredients in certain baked goods are illustrated below.

Bread and Biscuits

The essential ingredients in bread are flour, yeast, salt, and water; optional ingredients include sugar, malt, milk, shortening, enzymes, vitamins, chemical dough improvers, mold inhibitors, and minerals. High-heat NFDM and dairy blends can improve the nutritional profile and eating quality of yeast-leavened bread (Swanson, 2004). High-heat NFDM, which is normally used by bakers, influences water absorption in dough and physical properties of bread. Other quality factors in processing are mixing requirements, yeast fermentation rate, bromate activity in flour, and baking time and temperature.

High-heat treatment of skim milk at 88°C (190°F) for 30 minutes is employed in the manufacture of high-heat NFDM. It results in denaturation of milk proteins, which in turn enhances water absorption properties to provide a loaf of bread with good volume. The water-holding capacity of NFDM enhances the shelf life of bakery products. The proteins of NFDM aggregate at the air-water interface and reduce surface tension to produce stable emulsions. Encapsulation of air bubbles results in stable and elastic films in cakes.

To avoid direct contact of water with dry milk and prevent lumps in the dough mix, dairy blends are generally heaped at the top of flour in the dough mixer. Based on flour weight, up to 6% NFDM historically has been incorporated to simulate use of all the liquid ingredients in the formula. Dry milk tends to strengthen dough, thereby necessitating a longer mixing time. Hydration of milk protein during fermentation tightens the dough.

The fermentation time and tolerance are enhanced by inclusion of nonfat dry milk in bread formulas. Milk ingredients furnish enzyme cofactors (such as ammonium ions) for yeast metabolism during fermentation.

The yeast growth is accelerated when dairy constituents furnish nutrients, and the accelerated growth in turn causes enhanced loaf volume. The enhanced fermentation tolerance allows the baker to produce bread of uniform quality from one batch to another in terms of volume, flavor, crumb firmness, crust and crumb color, and shelf life. The lactose contained in milk, whey, and bakery blends is responsible for the formation of the golden brown crust in bread.

In the sponge-and-dough process, dry milk is generally added at the dough stage (Chandan, 1997; DMI, 2003). It also can be incorporated in the sponge to compensate for flour's low protein content, excessive amylolytic activity, short fermentation tolerance, and poor strength characteristic. Dough containing NFDM is set at a higher temperature: 27.2°C (81°F) in the winter and 25.6°C to 26.1°C (78°F to 79°F) in the summer. Dough made with NFDM requires a recovery period that is two to three minutes longer in the overhead proofer as well as in the pan. Proof box temperature should not exceed 37.8°C (100°F) and the relative humidity should be slightly lower (Matz, 1996)).

The no-time bread dough method does not require long bulk fermentation for conditioning the wheat gluten. Oxidizing agents (bromate, ascorbic acid, or azodicarbonamide) are dough conditioning/strengthening additives. The addition of 4% to 6% NFDM to no-time dough was shown to produce bread quality equivalent to the bread derived from the conventional sponge-and-dough process (AACC, 1995). Milk proteins are amphoteric in nature and tend to buffer pH changes, thus enabling them to temper the effect of oxidizing agents to optimize loaf volume and quality of grain crumb. A standard procedure for assessing the baking quality of NFDM is available (AACC, 1995).

Incorporation of NFDM in bread formulations also results in their nutrient fortification (Chapter 16). High-quality protein, calcium, phosphorus, potassium, vitamins A, D, and B_{12}, riboflavin, and niacin are augmented in the bakery item.

Dairy blends are a combination of whey, caseinates, and NFDM. Dairy substitutes contain dairy ingredients plus soy, corn flours, and soy protein and are designed to display the functionality of NFDM at a lower cost. They are used in bread doughs at a level of up to 6% on a flour-weight basis, and their use often requires modification in formula and processing conditions. More yeast is needed to maintain fermentation and proofing time, and more moisture is added to allow for the hydration requirement of NFDM. The baking temperatures and times are also adjusted to avoid overbrowning the crust (Pyler, 1988). Table 18.2 gives some examples of formulations for various types of bread, rolls, and buns.

Butter can contribute tenderness by restricting development of the gluten network, which also extends the bread's shelf life. Butter can be used as a carrier of herbal flavor, and it can give an attractive appearance to bread when sprayed on the surface. The contribution of butter to biscuits is manifold, with premium flavor, clean mouth feel, and flakiness as the major benefits.

Crackers

Cracker dough is generally stiff because of its low moisture content. Its stiffness interferes with lamination and sheeting into thin layers; however, milk fat in butter reduces the stiffness, making the dough more plastic for lamination. Sodium bisulfate hydrolyzes the disulfide bridges of gluten, further facilitating lamination. Anhydrous milk fat can be sprayed on the cracker surface to enhance gloss and appearance. A typical formulation for crackers is shown in Table 18.3.

For processing, flour, salt, and baking powder are sifted into a bowel. Butter, milk, and egg are mixed in to make a stiff dough.

Table 18.2. Composition of dough for some types of bread, rolls, and buns.

Ingredient	Bakers % (flour basis)				
	Pan bread	Country style bread	Milk bread	Milk rolls	Buns
Flour	100	100	100	100	100
Sweetener solids	7.25	5.0	7.0	10.0	12.5
Yeast	2.75	2.5	3.0	5.0	7.0
Yeast food	0.5	—	0.375	0.5	—
Salt	2.1	2.5	2.0	2.0	1.0
Shortening	2.3	2.0	—	4.0	12.5
Honey	—	5.0	0	0	—
Butter	—	3.5	3.5	3.5	—
Dairy blend/NFDM	2.0	8.0	6.0	6.0	3.0
Emulsifier	0.5	—	—	—	—
Dough conditioner	0.5	—	—	—	—
Protease enzyme	0.25	—	—	—	—
Calcium acid phosphate	—	—	0.25	0.25	—
Preservative	0.20	—	—	—	—
Water	64.0	66.0	66.0	66.0	51.0
Malt	—	—	0.5	0.5	
Whole egg	—	—	—	—	7.5

Adapted from Pyler (1992), DMI (1996a), Edwards (2007)

Table 18.3. Formulation for cracker production.

Ingredient	% Usage level	Baker's %
Flour, bread	55.8	100
Salt	1.11	1.99
Baking powder	0.35	0.65
Butter	12.90	23.10
Milk, whole	19.84	17.95
Total	100	179.29

Adapted from Chandan (1997), DMI (1996a,b)

After kneading, the dough is rolled very thin (about 1/8-inch thick). It is then cut into squares or rounds and placed on parchment-lined baking sheets. The crackers are pricked with a fork and baked in a 204.4°C (400°F) oven for 10 minutes or until lightly browned.

Pastry and Laminated Dough

In laminated dough, butter is applied to the surface of rolled out dough, and the dough is then folded and rerolled. More butter is applied, followed by repeated folding and rerolling steps. In this process, butter layers are dispersed in between layers of dough. As the pastry bakes, the moisture of butter turns into steam, and CO_2 is generated from leavening, both of which are instrumental in the pastry's rise and layer separation. The volume or rise in the baked pastry is directly proportional to the solid fat content of the fat.

Some bakers believe that European style cultured butter containing no salt or coloring and a minimum of 82% butterfat performs better than regular butter in certain baked goods. Croissants made with European style butter display higher specific volume and better flakiness inside and outside the surface.

Cakes

Detailed discussions on cakes of all types are available in other publications (Pyler, 1988; Conforti, 2007). Pound cake, yellow cake, and chocolate cake belong to the butter or shortened category of cakes. In principle, to obtain a good cake, tenderizing agents must be properly balanced with toughening ingredients. Sugar, shortening, egg yolks, leavening agents, and chocolate act as tenderization agents, which must be counterbalanced with toughening/binding ingredients consisting of flour, egg whites, and milk solids. Milk or buttermilk provides flavor and nutrients, and is responsible for a velvety texture, creamy white crumb, and brown crust. Lactose of

NFDM helps to enhance the light golden color of a cake's crust. It modifies the structure of cake by decreasing the swelling of starch granules without affecting cake volume.

In certain cake batters (high ratio, Oakes, and continuous-mix type), the soluble protein of milk or WPC aids in trapping air during mixing to incorporate air nuclei in the aqueous phase (Chandan, 1997). In high-fat bakery products, the whey proteins of WPCs assist in distribution of fat in the dough. The emulsification action creates a smooth and silky crumb as well as higher finished volume. Because whey proteins are denatured during baking, they exhibit good water-binding functionality, leading to moistness, tenderness, and enhanced keeping quality. Certain WPC products with enhanced emulsifying functionality can replace egg products at considerable cost savings. Table 18.4 gives typical formulations of some cakes.

Muffins

NFDM gives strength and body to muffins, and is involved in the requisite flavor, moisture retention, and shelf life. A muffin formula uses 5% to 12% NFDM based on flour at 100%. Table 18.5 gives typical formulations for basic muffins and bran muffins.

For production of a basic cake muffin, the dry ingredients are mixed in a Hobart mixer (N-50) at low speed for one minute, the shortening and eggs are added and mixed at low speed for an additional minute, and then water is added and mixing is continued at low speed for one minute. The batter is poured into muffin trays and baked at 205°C (400°F) for 19 to 21 minutes.

For bran muffins, the dry ingredients are blended at low speed for one minute in the Hobart bowl; the shortening, eggs, honey, molasses, and half of the water (500 g) are added and mixed at low to medium speed for one minute, followed by addition of the remaining water and mixing for another minute. The raisins are then mixed in and the batter is dispensed into the muffin tray and baked 193°C (380°F) for 20 to 25 minutes.

Butter Cookies

Butter makes cookies moist and soft through the interaction of butterfat, other solids, air, and water (Chandan, 1997). Fat coats solid particles in cookie dough, enabling it to flow during baking. Butter provides the desirable flavor and mouth feel profile to cookies. Dry whey is widely used in cookies for its superior functional attributes of color and texture development. Table 18.6 shows typical

Table 18.4. Some formulations for white, yellow, and pound cakes to illustrate the use of nonfat dry milk.

Ingredient	White cake	Yellow layer cake	Pound cake
	Bakers %	Bakers %	Baker's %
Cake flour	100	100	100
Granulated sugar	136	100	100
Water	106	66	37.77
Whole egg, liquid	0	49	58.39
Egg white, liquid	60	—	—
Nonfat dry milk	9	8	5.29
Salt	3	2	2.00
Baking powder	6	4	1.09
Lecithin	0.3	—	—
Shortening	25	41	58.39
Vanilla extract	—	2	2.00
Total	445.3	372	364.93

Adapted from Chandan (1997), Cross (2007), DMI (2003)

Table 18.5. Muffin formulations.

Ingredient	Basic muffin		Bran muffin	
	% by weight	Baker's %	% by weight	Baker's %
Flour	32.92	100	—	—
Bread flour	—	—	13.82	50.0
Cake flour	—	—	5.18	18.75
Bran	—	—	8.64	31.25
Sugar	19.75	60.0	8.64	31.25
Baking powder	1.65	5.0	0.41	1.50
Baking soda	—	—	0.61	2.20
Salt	0.41	1.25	0.41	1.50
Nonfat dry milk	2.47	7.50	3.46	12.50
Molasses	—	—	10.37	37.50
Shortening	13.17	40.0	5.18	18.75
Whole eggs, liquid	9.88	30.0	3.46	12.50
Egg solids	—	—	—	—
Honey	—	—	5.25	19.00
Water	19.75	60.0	27.65	100.00
Raisins	—	—	6.92	25.00
Total	100	303.75	100	361.70

Adapted from Chandan (1997), Cross (2007)

Table 18.6. Suggested formulations of chocolate chip and sugar cookies.

Ingredient	Chocolate chip cookies		Sugar cookies	
	Weight %	Baker's %	Weight %	Baker's %
All-purpose flour	26.00	100	41.79	100
Chocolate chips	24.16	92.3	—	—
Butter	17.47	67.2	14.71	35.19
Sugar	11.05	42.5	22.82	54.60
Brown sugar	10.06	38.7	—	—
Eggs, liquid	7.86	29.9	5.59	13.37
NFDM, high heat	2.14	8.2	5.56	13.30
Water	—	—	8.35	19.98
Salt	0.53	2.0	0.34	0.81
Baking soda	0.43	1.7	—	—
Baking powder	—	—	0.14	0.33
Vanilla extract	0.30	1.2	0.70	1.67
Total	100.00	383.7	100.00	239.25

Adapted from Chandan (1997), DMI (2003)

formulations of chocolate chip and sugar cookies made with butter and NFDM.

To make chocolate chip cookies, butter is creamed into sugar, followed by addition of vanilla, eggs, and the dry ingredients. The ingredients are thoroughly blended and chocolate chips are then dispersed into the dough. Baking is at 190°C (374°F) for 8 to 10 minutes. For sugar cookies, butter is creamed with sugar, followed by addition of vanilla, eggs, and water. The dry ingredients are then added and blended and the dough is chilled for an hour. Next, it is rolled out to a half-inch thickness and cut into a round shape with a cookie cutter, and the cookies are baked like chocolate chip cookies.

Cheesecake

Cream cheese is softened at 55°F (12.8°C) or at room temperature. A 10-inch diameter spring pan is greased and graham cracker

Table 18.7. Suggested formulation for standard cheesecake.

Ingredient	% by weight	Baker's %
Cream cheese	55.2	100
Sugar	21.2	38.5
Whole egg	12.7	23.1
Sour cream	9.4	17
Graham cracker crumbs	1.2	2.2
Vanilla	0.3	0.5
Total	100	181.3

Adapted from American Institute of Baking (2010). https://secure.aibonline.org/subscribers/.../.etins/v20iss0.4.text. Accessed on October 11, 2010

crumbs are deposited at the bottom. Cream cheese and sugar are blended at low speed for three to four minutes. The eggs and sour cream are then mixed in at low speed for two to three minutes and the mixture is poured into the pan. The pan is baked at 300°F (149°C) for one hour and allowed to cool in the oven without heat for another hour. The cake is then cooled on a cooling rack for an additional hour, followed by refrigerated storage overnight. A suggested formulation for cheesecake is given in Table 18.7.

Lime Pie Filling

The starting material of lime pie filling is sweetened condensed milk, which is acidified at 4°C to 10°C (40°F to 50°F) with lime or lemon juice to pH 4.6. It is then poured into a pie crust and allowed to thicken. It is stored under refrigeration after the required viscosity is obtained.

Snack Foods

Snack foods may be described as foods consumed at parties and in between meals (Altomare, Kettunen, and Cante, 1992). Their flavor may be savory or sweet. Snacks include a wide array of foods including cookies, candy, cakes, pies, pizza, potato chips, tortilla chips, corn puffs and curls, nuts, pretzels, popcorn, puffed cereals, granola bars, dried fruit, fruit leather products, hot dogs and other meat snacks, yogurt, cereal tubes with cheese filling, cheese crackers, cracker sandwiches with cheese filling, cheese potatoes, cheese cubes, etc. Dairy ingredients form an integral part of many snack items.

The primary application of dairy-derived ingredients in snack foods is for seasonings, because they enhance color and flavor. Dry cheese and whey powder blends used as seasonings are significant sales items for ingredient suppliers. Snack foods comprise four major categories: salted, baked, confectionery, and specialty. The confectionery products are discussed in Chapter 19.

Snack Manufacture and Application of Cheese Powders

The manufacturing processes and incorporation of cheese seasonings in snacks are discussed below.

Cheese Crackers

Cheese crackers usually are made from fermented dough of acidic character to augment cheese flavor. The formulation of cheese crackers resembles that of soda crackers except that the fat and moisture of cheese must be accounted for. Comminuted natural cheese (blend of cheddar, Swiss, blue and other cheeses), enzyme-modified cheese, or a cheese substitute is incorporated into the dough along with 0.25% paprika and a little cayenne pepper for color and flavor attributes. Sometimes a premix of ground cheese and shortening is held in the fermentation room for 24 hours and incorporated into the fermented dough (Bawa and Sidhu, 2003). The dough is baked and salted.

Potato Chips

For the manufacture of potato chips, washed potatoes are destoned and peeled, cut into a

manageable size, sliced, and rewashed. The slices are fried at 182°C to 193°C (360°F to 380°F) until the moisture is reduced to less than 1.5%. They are then salted or dusted with cheese or dairy seasonings prior to packaging.

Tortilla Chips and Corn Chips

The process for fried corn chips is similar to that for tortilla chips. A softer corn is generated by prolonged cooking in additional water; the resulting *masa* contains 50% to 52% water. The texture of the *masa* is coarser than tortilla masa. The *masa* is extruded, cut into pieces, and fried at 205°C to 210°C (400°F to 410°F). The corn chips are then cooled and coated with seasoning and salt. Corn chips contain 34% to 40% oil, whereas tortilla chips contain 22% to 26% oil. Corn chips have a little more salt (1.5% vs. 1%), and the moisture content of both should be 1% to 1.2% (Snack Food Association, 1988; Chandan, 1997). The process for making corn chips is illustrated in Figure 18.2.

Collets

Collets are puffed, second generation snacks, popularly named cheese curls and cheese balls, that are extruded and then either fried or baked. They are made from corn meal which is continuously fed into the extruder, accompanied by a small quantity of water. The mixture is subjected to heat and pressure in the barrel, and the extruder auger transports the meal through the extruder. Water in the corn meal dough is turned into vapor from heat generated by friction, creating high pressure in the system. As the stream is forced between two rotating heads, a sudden pressure release results in the formation of a rope with a puffed texture. The rope is cut into the appropriate length with a rotating knife; the snack shape may be cylindrical or ball-like. The moisture content after extrusion ranges from 8% to 10%. These extruded collets are then fried in vegetable oil or baked. Prior to seasoning application, the collets are further dried for 4 to 6 minutes at 149°C (300°F). The dried collets are sieved to remove the fines and coated with cheese seasoning.

Cheddar cheese is a popular seasoning. The typical composition of a cheese slurry consists is 58% to 66% collets, 24% to 30% vegetable oil, 5% to 9% dry cheddar cheese powder, 3% to 4% dry acid whey, 1% to 4% cheese flavor concentrate, and 0.2% to 3% salt (Chandan, 1997). The slurry is prepared in a water-jacketed kettle equipped with mixers; hot water at a constant temperature is circulated in the jacket. A homogeneous slurry of the various ingredients is obtained by a recirculation pump. The slurry temperature is maintained at 48°C to 54°C (120°F to 130°F), and should not exceed 63°C (145°F).

Coating procedures consist of (a) dry application, in which the extruded product is sprayed with warm vegetable oil, followed by dusting with dry seasonings, or (b) the oils, flavors, salt, and spices are slurried in a tank and sprayed onto the extruded product in a tumbler. Collets are coated in a rotating drum designed for a uniform and positive flow of product down the length of the unit. Longitudinal flights turn the bed of the collets over while the liquid cheese slurry is dispersed inside the tumbler at the in-feed end.

Cheese Seasonings

Dairy- and cheese-based seasonings contribute desirable color and flavor to many snack items. Sour cream, onion or garlic, and cheese flavors are popular in coated nuts, crackers, potato chips, and extruded and puffed snack items. In addition, nacho, ranch and barbeque flavors are widely accepted flavors in which whey and other dairy ingredients constitute significant building blocks. Dry whey powders may constitute as much as 5% to 30% of cheese powders and blends,

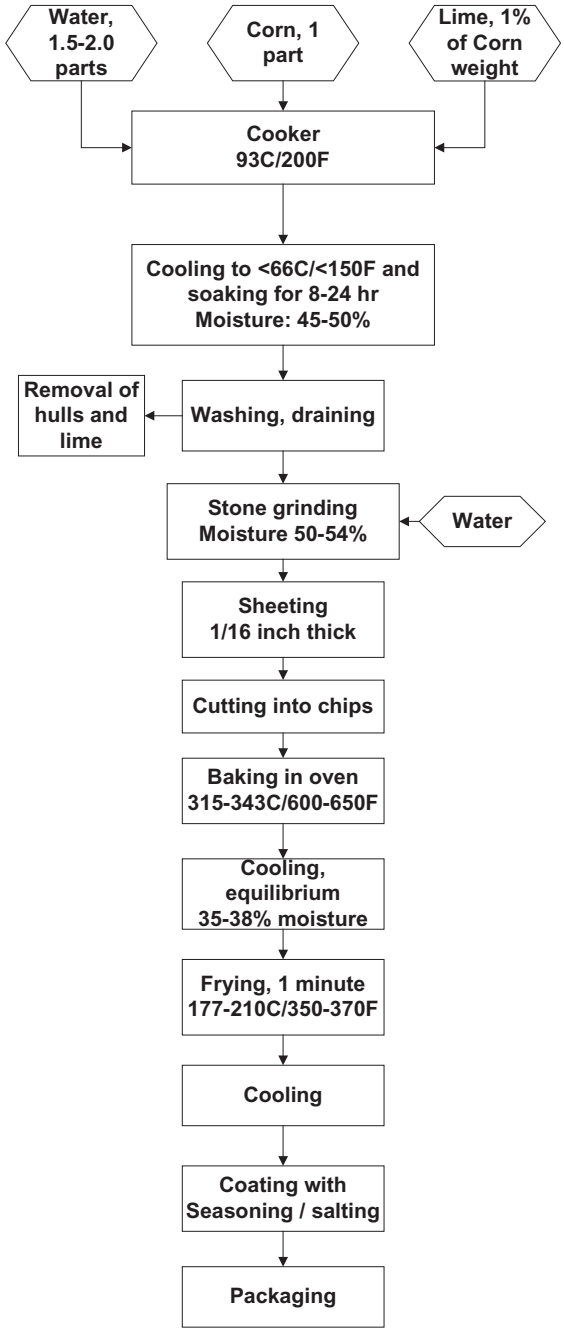

Figure 18.2. Process flow chart for corn chip manufacture.

and up to 50% of cheese seasonings (Johnson, 2000).

Cheese powders for snacks normally are developed specifically to satisfy the needs of snack manufacturers, and the custom design includes the spectrum of flavor notes. Frequently, salt is added to provide flavor and to act as a carrier of other flavors. It also functions as a flavor enhancer and modifier.

The original dried cheese blends were essentially dehydrated cheese manufactured by spray drying cheese slurry. Cheese seasoning may contain different levels of cheese. Cheese powders with high levels of cheese may contain greater than 90% cheese; blends may contain less than 50% to 70% cheese. The most common process involves blending the desired varieties of cheese and flavor with water, dairy-based ingredients, color, and emulsifying or melting salts to obtain a flavor concentrate. Emulsifying salts are commonly used to disperse the fat and protein components of the slurry prior to spray drying. The concentrate is then spray dried by atomizing it into a hot air stream and the powder collected. Ingredient suppliers have developed powders for specific applications. To reduce the cost of the seasonings, enzyme-modified cheese and butter flavor concentrates are frequently used along with whey solids to replace some of cheese solids. Certain nondairy ingredients are frequently included to save costs.

To provide a variety of flavors for the consumer, salty snacks are seasoned with flavor combinations of sour cream, pizza, taco, bacon, barbecue, salsa, onion and garlic. A sharper cheese-type flavor is achieved by the use of Romano, blue, parmesan and cheddar cheeses. If a mild cheese flavor is desired, Monterrey Jack or mozzarella cheese is used in the formulation. Cheese is used sparingly because it is an expensive component of cheese powder.

To contain costs, frequently cheese solids are replaced with flavor concentrates in the form of enzyme-modified versions of cheese or butter along with sweet or acid whey solids, buttermilk or skim milk solids, or vegetable oil. Whey powder possesses a clean and mild flavor of its own and lends itself very well to carrying cheese, oleoresin, and other savory flavors. These coat snacks uniformly to give them more volume and a desirable appearance. During baking, the components of dairy ingredients (NFDM, buttermilk solids, whey solids) interact with snack constituents to elaborate a battery of desirable flavors as well. The coating modifies the texture of snacks to give them smoother mouth feel. Whey proteins, prone to heat-setting on baking, provide structure to snacks.

Most salty snacks contain about 2% sodium chloride (Kuntz, 1996). For topical application, the salt size and form are important in dispersibility of the seasoning on the surface of the snack. Larger salt crystals dissolve slowly during eating the snack and provide a sharper lingering salt taste, as compared with small crystals. Appearance and adhesion properties also are influenced by the salt crystal size. Flavor enhancers such as monosodium glutamate and autolyzed yeast extract may be used as ingredients of cheese powder. Corn syrup solids (or maltodextrins) are used as carriers and impart flowability attributes.

Cheese powders with characteristic cheese flavor (cheddar, Romano, mozzarella, processed, blue, and parmesan) may be supplemented with stronger spice flavor to complement the flavor and appearance of the base. Certain herbs and spices lend themselves to be co-dried with the cheese slurry. Whenever there is loss or change in the character of the seasoning during drying, it is more appropriate to dry blend them with cheese powder.

Numerous flavor houses offer a variety of cheese seasonings compatible with various snack types; the formulation of seasonings is mostly proprietary. As an example, a typical specification for cheese powder includes

chemical-physical parameters which include moisture (3% maximum), fat (30% to 35%), salt (6% to 8%), color (25 to 30 Agtron units, green filter), and pH (5.0 to 5.5). The particle size distribution may be specified for adhesion attributes as passing 78% to 80% through U.S. #100, retained on U.S. #40 (2% maximum), and retained on U.S. #20 (1% maximum). Microbiological standards may be specified in terms of standard plate count of less than 50,000 CFU/g, coliform count of less than 50 CFU/g, and yeast and mold count of less than 50 CFU/g. In addition, the powder must be free of *Escherichia coli*, salmonella, *Staphylococcus aureus,* and *Listeria* contamination.

Seasoning formulations for application to various snacks are displayed in Table 18.8. The role and functions of various ingredients of seasonings are shown in Table 18.9. Examples of usage levels of cheese seasoning are given in Table 18.10. It is important to keep in mind that the usage level of a seasoning takes into account the rate of

Table 18.8. Suggested formulation of four seasonings.

Ingredient	% by weight			
	Nacho flavorings for corn and tortilla chips, popcorn, and crackers	Ranch-style for corn- based snacks	Seasoning for extruded snacks	Sour cream-onion for potato base snacks
Romano cheese, dried	35.0	—	—	—
Parmesan cheese, dried	10.0	—	—	—
Cheddar cheese, dried	5.0	2.0	68.0	—
Salt, flour, or mixture	18.9	20.0	6.2	12.0
Maltodextrin	17.6	10.0	—	—
Tomato powder	5.0	3.0	—	—
Monosodium glutamate (fine)	3.0	5.0	4.0	5.0
Onion powder	1.5	3.5	—	—
Citric acid (fine crystal)	1.0	0.8	—	1.0
Mustard	1.0	—	—	—
Garlic powder	0.5	1.2	—	—
Caramel powder	0.5	—	—	—
FD&C yellow #5 lake 41% dye	0.5	—	—	—
FD&C yellow #6 lake 41% dye	0.2	—	—	—
Red pepper, ground	0.3	—	—	—
Sour cream powder	—	—	—	25.0
Dry sweet whey	—	12.0	19.5	25.0
Nonfat dry milk	—	—	—	10.0
Dextrose	—	—	—	10.0
Parsley, dried	—	—	—	1.5
Free flow agent	—	—	—	0.5
Dry buttermilk	—	16.0	—	—
Corn starch	—	10.0	—	—
Shortening powder	—	5.0	—	—
Lactic acid	—	1.0	0.3	—
Disodium phosphate	—	1.0	—	—
Anti-caking agent	—	0.5	—	—
Paprika	—	0.5	—	—
Enzyme-modified cheese powder	—	—	1.0	—
Enzyme-modified butter oil	—	—	1.0	—

Adapted from Chandan (1997), Johnson (2000)

Table 18.9. Ingredients and their roles in cheese powder and seasoning formulation.

Ingredient used	Functionality
Cheeses	Flavor quality and intensity
Salt	Taste and adhesion of seasoning to snack
Corn starch	Adhesion of seasoning
Spices: onion, garlic, pepper, mustard	Variety of flavors
Citrates/phosphates	Cheese-emulsifying salts, slurrying agents
Lactic acid	Acid flavor
Enzyme-modified cheese and enzyme-modified butter oil	Flavor booster/rounded flavor
Maltodextrins	Carrier, no-flavor impact/flow agent
Corn syrup solids	Sweet rounded flavor/carrier/flow agent
Whey/whey products/lactose	Filler/flavor carriers, dairy flavor, cost control, effective coating
Vegetable oil	Adhesion/filler
Monosodium glutamate/autolyzed yeast extract	Flavor booster
Citric acid/sodium citrate	pH adjustment
Color	Appearance improvement
Tomato powder	Color and flavor
Sodium silicoaluminate/silica dioxide/magnesium carbonate	Flow agents (anti-caking)
Propylene glycol/wetting agent	Antidust agent

Adapted from Chandan (1997), Johnson (2000)

Table 18.10. Typical usage levels of cheese seasonings in various snacks.

Snack	Application mode and amount		
	Topical	Oil slurry*	Incorporated in the flour dough
Popcorn	6% to 12% weight of popcorn	22% to 29% weight of popcorn	—
Potato chips	5% to 8% weight of chips	—	—
Corn collets/balls	6% to 12% weight of collets/balls	22% to 29% weight of collets/balls	Approx. 10% weight of flour
Cracker dough	—	—	8% to 10% weight of flour
Cracker filling	—	—	30% to 35% weight of filling

*55% to 60% oil, 40% to 45% cheese powder
Adapted from Chandan (1997)

application as well as mode of incorporation in the snack. Table 18.11 gives typical specifications of finished snack products.

To guard against deterioration of oil in fried snacks, only compatible seasoning flavorings are used. Oil-soluble flavors are preferred over water-soluble flavors. Seasoning applied to the surface of unbaked snack crackers must be resistant to heat to avoid heat-induced interactions and volatilization. In cheese crackers, the cheese preparation is incorporated in the dough. Fried and oiled

Table 18.11. Typical finished product specifications of some extruded snacks.

Component	Baked collets	Fried snack
% Moisture	1.2–1.6	1.4
% Salt	2.2	2.2
% Oil content	33–37	36
% Cheese content	5.4	7.2
Cheese ball, diameter	—	0.625 inch

Adapted from Chandan (1997)

snacks are coated or dusted with seasonings in a tumbler to continue dispersion of the seasoning. Another method commonly used in the industry involves slurrying the powder in oil and spraying the slurry onto the snack in a rotating tumbler. Because the oil-seasoning slurry is drawn from a holding tank, bulk density, particle size and shape, and sedimentation rate of cheese powder are important factors in avoiding plugging of spray nozzles and assuring uniformity of coating on the snacks. It is important to optimize the surface adhesion of the seasoning by designing spray equipment appropriately.

The mesh size of the seasoning powder should be compatible with the oil content of the snack. For potato chips with an oil content of 36% to 38% by weight, a relatively coarse particle size of 40 to 100 mesh is adequate for optimum adhesion. Tortilla chips with lesser oil (18% to 22%) require a much finer particle size. In low-fat snacks it may be necessary to form a surface film to facilitate adhesion of the seasoning. The film is created by spraying a solution of gum acacia, corn syrup solids, or maltodextrins onto the surface of the snack and drying at 121°C (250°F) in convection heating equipment. In some cases, a superior flavor profile can be achieved by using cheese powders in the formulation of dough, followed by topical application.

The color of cheese powders ranges from white to deep orange, depending on regional or manufacturer's preferences. The seasoning color imparts a desirable color to the snack that is indicative of the flavor. Colors may be of vegetable origin (turmeric, paprika, beet, annatto, and beta-carotene) or of synthetic origin (Food Drug & Cosmetic; FD&C-permitted colors). They should make the product bright and exhibit stability during its shelf life.

The nutritional contribution of cheese powders to snacks is generally marginal if the application rate is low, such as 10%. However, at higher levels, whey powders contribute high-quality protein and calcium to the snack. Quality control tests for snacks involve organoleptic evaluation and determination of moisture, oil, salt, and amount of cheese coating. Other tests that are frequently performed concern color and bulk density.

Cheese and Dairy Sauces

Sauces derived from cheddar and other types of cheese constitute a significant but specialized business for ingredient suppliers. Their popularity is due to their convenience of use for food preparation in food service and fast food operations. Main applications include preparation of sandwiches, omelettes, nachos, pasta dishes, and as a toppings for potatoes and vegetables. There are wide variations exist in cheese sauce flavors, including mild and aged cheddar and Mexican food flavors such as salsa, nacho, and jalapeno.

Processing

Cheese sauces are generally manufactured by a low-acid thermal processing and aseptic packaging system. The manufacturing procedures are mainly in the proprietary domain of private companies. A primary innovation in cheese sauce relates to development and retention of desirable flavor, body, and texture during "commercially sterile" heat processing temperatures and times. Table 18.12 shows some patented formulations and processes for shelf-stable cheese sauce.

Cheese sauce processing begins with shredding the cheese in a grinder and conveying it into a processing kettle. Water, emulsifying salts, and other dry ingredients are incorporated with agitation to form a slurry for aseptic processing and packaging. A typical processing system consists of a high-shear blender, product surge tanks, balance tanks, a homogenizer, a tubular heat exchanger preheater, four scraped-surface heaters, a holding tube, 12 scraped-surface

Table 18.12. Formulation of cheese and dairy sauces.

Ingredient	Cheddar cheese sauce*	Low-cost cheese sauce**	Low-solids cheese sauce***	Creamy cheese sauce****
Grated cheddar cheese	11.8	5.0	—	—
Monterrey cheese	—	—	12.9	—
Enzyme modified cheese flavor	0.6	—	1.3	—
Disodium phosphate	0.5	3.5	0.7	—
Modified waxy maize starch	6.0	—	5.6	2.0
Waxy starch, unmodified	—	—	1.4	—
Locust bean and guar gum	0.3	—	—	—
Fillers and gums	—	8.8	—	—
Carrageenan	0.06	—	—	—
Lactic acid	0.05	0.33	—	—
Dairy solids (NFDM)	—	10.0	—	—
Whey protein concentrate 34	0.2	—	—	—
Vegetable oil/fat	5.5	18.0	7.4	—
Maltodextrins (5 DE)	—	—	5.3	—
Monosodium glutamate	—	—	0.35	—
Sodium citrate	—	—	0.3	—
Citric acid	—	—	0.05	—
Acetic acid	—	—	0.05	—
Sodium hexametaphosphate	—	—	0.1	—
Emulsifier, monoglyceride	—	0.3	0.1	—
Onion powder	0.3	—	—	—
Garlic powder	0.03	—	—	—
Spices and seasoning	0.2	—	0.03	—
Flavor and spices	—	6.41	—	—
Yellow coloring	0.03	0.27	0.25	—
Sodium chloride	0.8	1.0	1.3	0.7
Water	74.0	36.2	—	81.7
Condensate		10.0	—	
Olive oil	—	—	—	8.0
Xanthan gum	—	—	—	0.4
Egg yolk	—		—	1.0
Cheese blend (mascarpone, low-lactose parmesan, pecorino)	—	—	—	5.0
Cream, 40% fat	—	—	—	6.0
Polyphosphate	—	—	—	0.2
Sorbic acid	—	0.1	—	—
Cultured dextrose (preservative)	—	0.5	—	—
Nisin	—	0.05	—	—

Adapted from Spanier (1986), Caro, Motala and Wagner (2008), Duval, Kruhmar and Ratcliff (1994), Gamay et al. (2009)

*Mix starch, carrageenan, guar, and locust bean gum to form slurry and cook to 76.7°C to 98.9°C (170°F to 210°F) to form a paste. In another vessel, mix cheese, lactic acid, cheese flavor, salt, dairy protein, phosphate, and remaining ingredients, heat to 60°C to 79.4°C (140°F to 175°F) and homogenize. Blend the gum paste and package in containers and retort for sterilizing the sauce.

**Cheese solids, cheese flavor, and all other ingredients are mixed in a steam cooker and blended with the oil/fat by heating/steaming and pasteurized at 71.1°C (160°F). The sauce is homogenized and hot filled into polyethylene flexible pouches lined with aluminum foil laminates, plastic, ceramic, or glass containers. The sauce is shelf stable for a year and no sterilization treatment is necessary.

***The salts, including phosphates, are added to water in a jacketed vessel and heated to 71.1°C to 93.3°C (160°F to 200°F) under agitation. Cheeses are shredded and blended. The emulsifier and fat/oil are added, followed by the starches, maltodextrins, and all other ingredients. The mixture is homogenized at 76.7°C (170°F) and 1,700 psi (first stage) and 500 psi (second stage). The sauce is packaged hot in glass jars and retorted at 121.1°C to 137.8°C (250°F to 280°F) for 30 to 45 minutes. The rotation is 10 to 20 rpm. The sterilized sauce may contain vegetables and other foods.

****Prepared by mixing all of the ingredients except the cheese and cream, homogenizing the mix, adding the grated cheese and cream, blending and packaging in glass jars at room temperature, and subjecting to rotary sterilization.

coolers, and aseptic filler for No. 10 cans or appropriate pouch-filling equipment.

Cheese sauce ingredients are mixed in the high-shear blender and the slurry is pumped into surge tanks equipped with agitation and temperature control. The slurry is fed into the balance tank where its temperature is raised to 43.3°C (110°F) with a heat exchanger using energy from the condensate from the heaters. The warm slurry is then homogenized and preheated to 76.7°C (170°F) by the tubular unit prior to entry into scraped-surface heaters and the holding tube. Here, the sauce is heated quickly to 138.9°C (282°F) and held for 21 seconds to achieve commercial sterility. Cooling of the sterilized product to 37.8°C (100°F) is achieved quickly by running the sterilized sauce through 12 scraped-surface coolers. The flavor quality is controlled by precise time-temperature control during heating and cooling cycles. The cheese sauce is dispensed and sealed aseptically in appropriate containers.

The canned product is held for 14 days at the manufacturing plant for quality control clearance. Samples drawn from manufacturing runs are incubated at 37.8°C (100°F) for two weeks. Quality checks include integrity of can seams and vacuum, pH, color, standard plate count, and thermoduric bacteria count. Aseptically processed cheese sauces require no refrigerated storage until they are opened. After opening, the sauce should not be held longer than 10 days at 4.4°C (40°F). The sauce is pumpable and provides good portion control in a fast food operation. Versatility of flavor and thick or thin body attributes can be formulated. A skin may form on the surface of cheese sauce that is exposed to air or steam. This can be avoided by covering the can with aluminum foil and stirring occasionally.

Quality Evaluation

Sensory evaluation of cheese sauce is conducted in the as-is form. Sauces also are tested as a topping on potatoes or nachos or in macaroni and cheese and other pasta products. Occasionally, its flavor performance is tested in cheese sandwiches. Desirable attributes of appearance include uniformity of color, shiny appearance, and smooth texture. Defects include dull appearance, streaking, specks, and rough texture. The flavor should be fresh and clean with no detectable canned, bitter, salty, or sweet flavor notes. The texture should be smooth and creamy with no perception of graininess, lumpiness, or gumminess.

Dairy Salad Dressings

Spoonable and pourable dairy dressings displaying rich creamy mouth feel are based on buttermilk or cultured milk. Regular salad dressings contain 30% to 80% fat, and mayonnaise is 65% fat. Dairy-based dressings may contain 0% to 12% fat. They must to be refrigerated in storage distribution and use. Flavors include blue cheese, creamy thousand island, avocado, creamy garlic, creamy Italian, and creamy French.

Production of the dairy base involves formulation of the base preparation similar to that of cultured buttermilk. Stabilizers and emulsifiers are added to the cultured base, followed by pasteurization and homogenization. The base is then blended with salad bases to obtain the dressing.

Figure 18.3 shows a process flow sheet for dairy salad dressing manufacture.

Processed Meat Products

Dairy ingredients are used as extenders in meat products. During manufacture of processed meat products, a key objective is the formation of a stable heat-induced gel (emulsion) in which water and fat are bound to yield a palatable and attractive product. A meat emulsion is a multi-phase system. Solid fat particles, muscle fibers, and connective tissue are dispersed in the aqueous phase

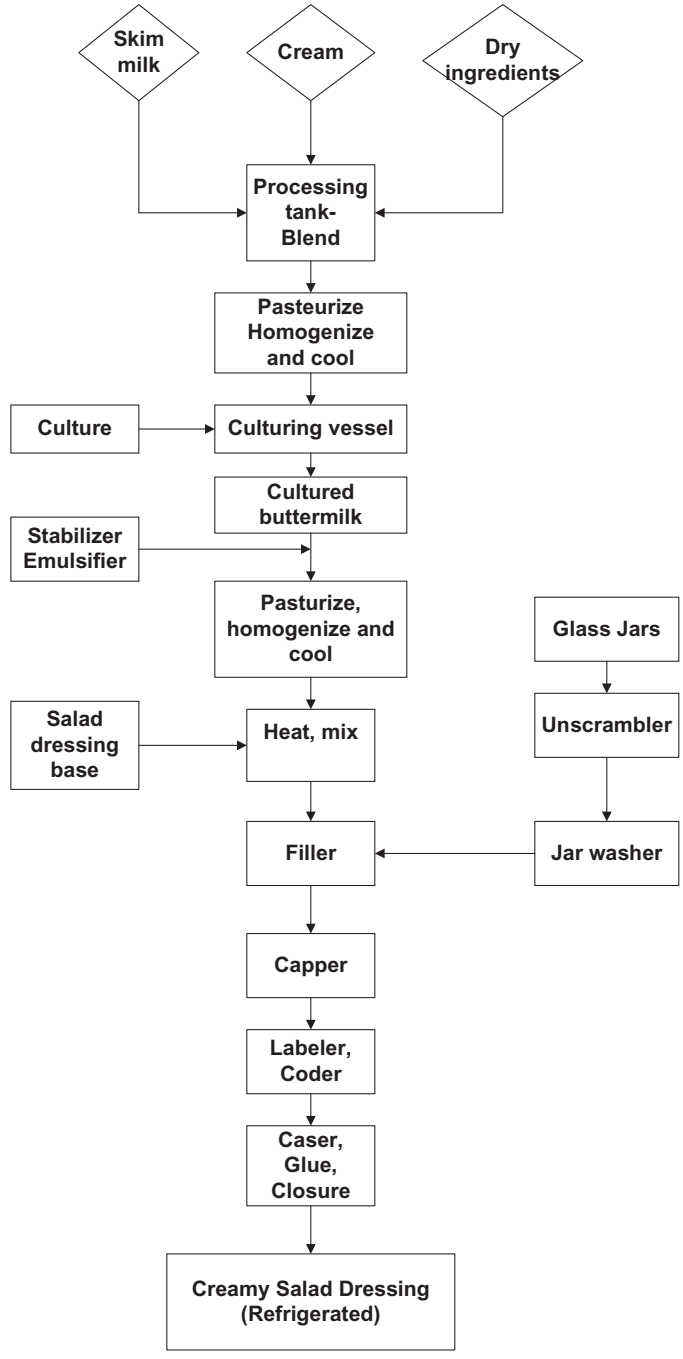

Figure 18.3. Flow diagram for creamy salad dressing manufacture.

(continuous phase), containing soluble salts and proteins. Emulsion stability is achieved by dispersing fat as fine particles and creation of an interfacial protein membrane at the fat-water interface. The fat, muscle, and connective tissue are converted to fine particles by grinding.

The comminuted meat consists of individual meat particles interspaced in the water medium containing protein capable of displaying adhesion properties. Accordingly, proteins perform an important structural and functional role. The functional attributes are water binding (protein-water interaction), fat binding (protein-lipid interaction), meat binding (protein-protein interaction), viscosity, solubility, gelation, and surface activity.

Dairy ingredients can be used as functional ingredients in the manufacture of processed meat, poultry, and seafood. In these cases, lactose and milk proteins are generally used.

The application of dairy ingredients is determined largely by the degree of comminution of meat in the formula. Hams and bacon do not contain comminuted meat during processing. Sausage, bratwurst, hamburgers, meat patties, meatballs, and chicken nuggets are obtained from coarsely comminuted meat. Frankfurters, hot dogs, bologna, meat loaves, and liver sausages are derived from finely ground meat.

Lactose

Lactose use in processed meats limited to pasteurization treatment enhances the intensity and brightness of color. In sausage manufacture, lactose at the 1% to 2% level assists in controlled browning (Maillard reaction) after frying or microwave heating (DMI, 1998). In the fermented sausage process, lactose (1% to 3% usage level) furnishes the carbohydrate necessary for the growth and production of lactic acid by starter cultures. Lactobacilli and Pediococci grow well when lactose is present in the medium. The resultant reduction in pH discourages the growth of undesirable organisms and exerts a preservative effect.

In the manufacture of reformed hams, lactose use varies from the 0.5% to 2% level. It improves the water-holding capacity in pumped ham and other coarse or noncomminuted meats. In the finished meat product, lactose (1.2%) along with salt (2% to 3%) and phosphate (0.3% to 0.5%) helps in extraction and swelling attributes of meat protein. Lactose at the 2% to 3% usage level masks the bitter after taste associated with salt and phosphate. Lactose also masks the strong liver taste associated with liver-based patés and spreads. Lactose enhances the cured color of ham, and it improves the sliceability and yield of cooked ham, cooked sausages, and liver products (Chandan, 1997). Lactose-containing products such as whey and NFDM are functionally inferior because they contain too much calcium, which interferes with the binding properties of meat proteins.

Milk Proteins

Milk protein contributes to the stability of the emulsion. Milk protein or milk protein hydrolyzates can be used at the 0.8% to 1.6% level. Sodium caseinate is widely used because it competes favorably with meat proteins for absorption at the oil-water interface to stabilize fat in the emulsion.

Other important functional properties of sodium caseinate in meat systems are that it does not gel on heating, the denatured form improves functionality, and it does not contribute to the development of viscosity. The addition of milk proteins in frankfurters decreases the ingredient cost and increases the yield (Toldra and Flores, 2007).

Nonfat dry milk at the 2% level boosts the overall quality (better sliceability, color, and flavor) of cooked turkey deli breast meat. NFDM can be used at the 3% to 5% level in bologna, liver sausage, cotto salami, and

meat loaf, and at the 9% to 12% level in corned beef loaf and roast beef loaf. Evaporated milk can be blended in sweet and sour meatballs at the 18% to 19% level. In smoked sausage, use of 2.5% NFDM and 1.25% whey protein concentrate (80% protein) increases the water-holding capacity and yield and provides better emulsification and enhanced adherence attributes (DMI, 2003).

Whey products contain high-quality protein and calcium. Their use may improve the nutritional status of meat products such as bologna, reduced-fat sausage, and roast beef (DMI, 1996). Whey protein concentrates optimized for emulsification can facilitate fat distribution in processed meat products and their use improves salt tolerance. Furthermore, whey protein acts as a stabilizer of the emulsion. In lunch meat and sausage, whey ingredients function as adhesion agents in binding meat pieces after chopping and blending with other ingredients. Gelation of whey protein, induced by further heating and salt treatment, entraps more water in the matrix and creates a strong gel net-work. Accordingly, moisture is held tightly and syneresis is prevented, and the yield of meat products is increased. The water-binding functionality of denatured whey protein improves the texture of meat products in that they are perceived to be more moist and fresh. Surimi, pressed ham, and bacon are application areas of the whey protein ingredients.

Processing Considerations

Milk proteins for use in meat processing must be in a hydrated state to achieve the best functionality. Accordingly, the powder should be blended with chopped meat prior to addition of ice or water. Another procedure involves slurry preparation of one part of milk protein to six parts of water in a chopper and incorporation of the slurry during the communition process. Alternatively, a dispersion of five parts of fat, one part of milk protein, and five parts of water may be prepared and blended with the meat emulsion at the 10% to 25% level.

Temperature control during meat processing is important to the stability of meat emulsion. Normally, it should not exceed 18°C (64.4°F). Because milk proteins have a wider temperature tolerance, milk proteins added to meat permit a wider range of temperature.

Poultry rolls and bolognas as well as chicken nuggets and patties acquire a firmer texture and have improved sliceability and juiciness when sodium caseinate is used as an ingredient. A pre-emulsion consisting of one part of sodium caseinate, five parts of chicken skin, five parts of chicken fat, six parts of water, and two parts of ice is prepared and is subsequently incorporated in the product at the 10% level.

Turkey breast production includes sodium caseinate and milk protein concentrates to increase yield from 3% to 6%. Usage levels of milk protein range from 0.8% to 1.5%.

Seafood processing, especially that of tuna, can benefit in terms of juiciness and a 6% to 8% increase in drain weight from the use of milk protein hydrolyzate. A suggested formula for water-packed tuna employs 75% steam-cooked tuna and 25% brine. The brine contains 86% water, 4% salt, and 10% milk protein hydrolyzate (Van den Hoven and Van Valkengoed, 1992). Whey protein concentrates and starch added at the chopping stage improve the water-binding capacity and juiciness of fish sticks, fish nuggets, and fish pastes (de Wit, 2003).

Functional Foods

Functional foods may be defined as foods containing significant levels of biologically active components which impart health benefits beyond basic nutrition. They are also referred to as wellness foods, healthful foods, or nutraceuticals. It is now scientifically accepted that foods play a role in the preven-

tion of diseases such as cancer, coronary heart disease, atherosclerosis, stroke, diabetes, and liver ailments (Jackson and Paliyath, 2007).

Dairy Ingredients as Functional Ingredients

Milk has been described as nature's nearly perfect food because it provides vital nutrients including proteins, essential fatty acids, minerals, and lactose in balanced proportions. Milk and milk products are recognized as important constituents of a well-balanced and nutritionally adequate diet. Milk and dairy foods complement and supplement nutrients available from grains, legumes, vegetables, fruits, meat, seafood, and poultry.

The dairy industry has provided the consumer with choices of low- and fat-free ingredients. The functional properties of various milk constituents have been reviewed elsewhere. (Chandan, 1999, 2007, 2008; Chandan and Shah, 2006, 2007; Chandan and Kilara, 2008).

Bioactive Peptides

Functional peptides are generated during digestive processes in the body and during the fermentation processes used in fermented dairy foods. They arise from casein and whey proteins. These peptides are inactive in the native proteins but assume activity after they are released. They can be absorbed in intact form to exert various physiological effects locally in the gut or they may have a systemic effect after entry into the circulatory system. The reader is referred to chapters 12 and 16 for details. Casomorphins and lactophorins derived from milk proteins are known to be opioid agonists, whereas lactoferroxins and casooxins act as opioid antagonists. The opioids have analgesic properties similar to aspirin. Casokinins are antihypertensive (lower blood pressure), casoplatelins are antithrombotic (reduce blood clotting), immunopeptides are immunostimulants (enhance immune properties), and phosphopeptides are mineral carriers.

Casein phosphopeptides may aid in bioavailability of calcium, phosphorus, and magnesium for optimum bone health. They also may be helpful in preventing dental caries and may have a role in secretion of enterohormones and immune enhancement. The role of casein peptides in regulation of blood pressure is showing promise. Conversion of angiotensin-I to angiotensin-II is inhibited by certain hydrolyzates of casein and whey proteins. Because angiotensin-II raises blood pressure by constricting blood vessels, its inhibition causes lowering of blood pressure. This ACE-inhibitory activity would therefore make dairy foods a natural functional food for controlling hypertension. Several whey products containing discreet bioactive peptides are commercially available.

The glycomacropeptide (GMP) released from κ-casein as a result of proteolysis may be involved in regulating digestion as well as modulating platelet function and thrombosis in a beneficial way. It is reported to suppress appetite by stimulating the CCK hormone. Consequently, it may be a significant ingredient of satiety diets designed for weight reduction. Furthermore, this peptide may inhibit binding of toxins in the gastrointestinal tract. As an active ingredient, the GMP is commercially available as a fractionated whey product.

Probiotics

Probiotics are food or supplements containing concentrates of defined strains of living microorganisms that exert health benefits beyond inherent basic nutrition upon ingestion in certain doses. This definition stresses the importance of ingestion of several hundred millions of live and active microbial culture. Various strains exert health benefits but specific strains are important if health claims are made. The health benefits of specific strains of probiotics are enumerated in Table 18.13.

Table 18.13. Specific strains of probiotics and their documented benefits.

Strain	Benefit reported in human trials
L. acidophilus NCFM	Lactose digestion, reduces bacterial overgrowth in small intestine
L. acidophilus(CUL60)+ B. bifidum (CUL20)	Reduces fecal toxin of *C. difficile*
L. casei DN114-001	Enhances immune function
L. casei Shirota YIT9029	Enhances immune function, balances intestinal microbiota, combats recurrence of superficial bladder cancer
L. helveticus R0052 + L. rhamnosus R0011	Controls diarrhea in children, eradicates *Heliobacter pylori* infection
L. johnsonii La1/Lj1	Enhances immune function, eradicates *Heliobacter pylori* infection
L. plantarum 299V	Relieves irritable bowel syndrome, restores post surgical gut nutrition
L. reuteri SD2112	Controlling diarrhea, improving immune function
L. reuteri RC14 + L. rhamnosus R0011	Controls diarrhea in children, eradicates *Heliobacter pylori* infection
L. rhamnosus GG	Prevents children's infectious diarrhea and atopic dermatitis, enhances immune function.
L. salivarius UCC118	Alleviates inflammatory bowel disease
B. animalis DN173-010	Normalizes intestine transit time
B. infantis 35624	Alleviates irritable bowel syndrome
B. lactis BB-12	Enhances immune function, alleviates diarrhea in children
B. lactis HN019 (DR10)	Enhances immune function in the elderly
B. longum BB536	Alleviates allergy symptoms, balances microbial ecology
VSL #3 (blend of S. thermophilus, four strains of *lactobacillus* and three strains of *bifidobacterium)*	Alleviates inflammatory bowel conditions
S. thermophilus (many strains)	Aids in lactose digestion
Sachharomyces cerevisiae (boulardii) lyo	Alleviates antibiotic-induced diarrhea and *C. difficile* infections

Adapted from Sanders (2007), Chandan (1997)

Supplementation of probiotics with prebiotics can be create very effective functional foods. For example, fructo-oligosaccharide (FOS) and galacto-oligosaccharide (GOS) are exclusively used by a few strains of *Bifidobacterium bifidum* and *Lactobacillus acidophilus*. Thus, a combination of FOS or GOS along with these cultures is likely to induce the proliferation of these cultures in preference to other microflora in the gastrointestinal tract. For more information on functional foods and probiotics, see other publications (Chandan, 1997, 1999; Chandan and Shah 2006, 2007).

Dairy Biologics

Bovine immunoglobulins are a new class of oral therapeutics that are emerging dairy ingredients and food supplements. They are comprised of antibodies from colostrum of dairy cows and are designed to attack infections in the gastrointestinal tract of humans. They are consumed orally and provide passive immunotherapy. Cows are immunized against specific human pathogens during the dry period, thereby effecting short-term increases in specific antibodies. The process starts from identification of a specific pathogen, immunization of pregnant cows, and collection of four days of postpartum milk, followed by processing and formulation for site-specific delivery. The following table shows some biologic products containing specific antibodies.

Advantages of the immunoglobulin biologics include safety, ability to target specific pathogens, and versatility of action against viruses, fungi, parasites, bacteria, and toxins. In addition, polyclonal antibodies contained in the biologics bind multiple target sites.

Table 18.14. Specific antibodies of certain biologics.

Disease	Antibody against:
Diarrhea	Enterotoxigenic *E.coli* Rotavirus *Shigella flexneri* Cholera *Clostridium difficile* *Cryptosporium parvum*
Tooth decay	*Streptococcus mutans*
Ulcers, gastritis	*Helicobacter pylori*
Thrush	*Candida albicans*

Regular bovine colostrum contains antibodies against many human pathogens including *Candida albicans, Cryptsporium parvum, Escherichia coli, Escherichia coli.J5, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia marcescens HY, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptoccus faecalis, and Streptococcus viridians.*

Colostrum is recognized for its immune factors and growth factors. In the gastrointestinal tract, bovine colostrum may have a role in maintenance of integrity of mucosa, permeability, local immunity, systemic immunity, and antigen handling. A commercial colostrum powder containing 40% IgG and other immunoglobulins is marketed as a food supplement. The product is claimed to aid in healthy nutrition and cell repair/regeneration. The beneficial attributes include anti-inflammatory effects. It is claimed to promote enteric health, immune health, and cognitive ability. Additional application of colostrums is in sports nutrition and in skin care products and cosmetics.

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Chapter 19

Dairy Ingredients in Chocolate and Confectionery Products

Jorge Bouzas and Steven Hess

Confectionery is the collective term applied to edible products usually compounded of sugar as a common ingredient. Candies are often combinations of several confections, with chocolate used as coating on candy bars, ice cream, cookies, and nuts (Bouzas and Brown, 1995).

Chocolate and chocolate confectionery together account for about 60% of sales of the confectionery industry worldwide. In most countries, legal requirements for the composition of chocolate must be met for the product to be labeled as chocolate. If these requirements are not met, the product must be labeled using an alternate name such as chocolaty candy or chocolate-flavored candy. In the United States, chocolate and cocoa are standardized by the U.S. Food and Drug Administration, which prescribes the quantitative elements and consequently, some of the qualitative aspects as well (Anon., 1993). Internationally, the Codex Alimentarius provides standards for cocoa products and chocolate (Anon., 1998). Many European countries follow Codex standards for chocolate. Most other countries either follow Codex standards or have specific standards of their own, which are often similar to either the Codex or U.S. standards.

Milk and other dairy ingredients are essential ingredients in chocolate and confectionery products, in part because they help to provide the flavor and textural experience consumers expect, but also, if the correct dairy ingredients are present in the required amounts, they allow a given product to meet local standards of identity.

Chocolate Confectionery

The most popular chocolate consumed worldwide is milk chocolate, which contains at minimum cocoa mass, sugar, cocoa butter, and milk. A typical formula for a milk chocolate is shown in Table 19.1. The following section presents a brief description of the typical milk chocolate manufacturing process. For more detailed descriptions of cocoa bean and chocolate processing, see Beckett (1999), Minifie (1989), and Lees and Jackson (1973).

Milk Chocolate Processing

The first step in chocolate manufacture is the mixing of cocoa butter, chocolate liquor (also known as cocoa mass), milk, and sugar. It is important to note that in the production of milk chocolate, water must be removed from the milk at some point during the manufacturing process. Chocolate with moisture content above about 2% is normally unacceptable because it has poor keeping quality, high viscosity and yield stress, and undesirable texture. Many milk chocolate producers use dry milk powder and other dairy components to prepare chocolate, whereas others

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Table 19.1. Typical milk-powder-based milk chocolate formula.

Ingredient	% (w/w)
Sucrose	46.60
Cocoa butter	20.00
Whole milk powder	21.00
Cocoa mass	12.00
Lecithin	0.30
PGPR	0.05
Vanillin	0.05

condense fresh whole milk with sugar and either dry this mixture to produce milk crumb or blend the mixture with chocolate liquor and then dry the resulting mixture, producing milk chocolate crumb (Bouzas and Brown, 1995). Additional aspects of both these so-called milk-powder-based chocolates and crumb-based chocolates are discussed in the next section.

The second stage in chocolate processing is a particle size reduction process known in the trade as refining, essentially a fine grinding operation in which the coarse paste is passed through a roller mill or media mill to reduce the particles of crystalline sugar, fibrous cocoa matter, and milk solids to a nominal diameter of 15 to 50 μm. The particle size of chocolate is extremely important to the overall quality of chocolate, hence the refining process which controls particle size is crucial (Beckett, 1999). A particle size greater than about 30 micrometers will result in a chocolate that is gritty in the mouth, whereas a particle size of less than around 18 micrometers can result in a chocolate with a very high viscosity and yield stress that can be difficult to handle during downstream processing.

The refined chocolate paste is then conched, a step critical to flavor and texture development. Conching is a high-shear mixing process that works chocolate flake and crumb into a fluid paste, embracing a wide range of phenomena including flavor development, reliquification of the chocolate paste, gloss development, destruction of particle agglomerates, and modification of the melting quality (Beckett, 1999). Because the conching process can sometimes employ temperatures as high as 70°C to 80°C and times as long as several days, flavor reaction products, such as Maillard reaction products, formed during this step contribute to the flavor profile of the finished chocolate. The composition of the dairy solids used in chocolate manufacturing has a strong influence on the development of the desired flavor profile during conching.

Emulsifiers and additional cocoa butter are often added at the end of conching to standardize the final product and adjust the flow properties of the fluid chocolate mass to the final specifications suitable for forming the desired finished product. Lecithin is by far the most common emulsifier used in the chocolate industry (Hoskin and Dimick, 1994), although the ammonium phosphatide known in the trade as YN is also allowed in some countries. In addition, polyglycerol polyricinoleate (PGPR) is also widely used in chocolate products. The amount of emulsifier employed typically falls within the range about 0.2% to 0.6% (Minifie, 1980). Flavoring materials are also added at this point, with vanillin and natural vanilla the most commonly added in the United States.

Conching and standardization is followed by tempering, a mixing and cooling process using specific temperature ranges to cause a small part of the liquid cocoa butter to be crystallized in the appropriate polymorphic form. These crystals act as nucleation sites when additional cocoa butter crystallizes during solidification of the chocolate, thereby preventing subsequent surface discoloration known in the trade as fat bloom. They also provide the appropriate texture to the finished product. Failure to produce the correct crystalline form not only results in problems for the manufacturer, but also gives a product without the gloss, snap, and color normally expected by the consumer (Bricknell and Hartel, 1999; Aguilar and Ziegler, 1993).

Milk in Chocolate Formulations

Milk in its various forms has been used as an ingredient in chocolate manufacture since the introduction of milk chocolate in 1876. Milk and milk solids are essential to flavor, color, and texture; they provide nutrition and bulk; and they contribute to gloss and shelf life (Campbell and Pavlacek, 1987; Kinsella, 1970).

Milk chocolate was traditionally made by using fresh fluid milk in the crumb process mentioned previously. The crumb process, however, requires a great deal of highly specialized equipment, and can therefore be very costly from a capital equipment standpoint. Therefore, in the last few decades, the chocolate industry has been increasingly moving toward production of powder using milk powder in the so-called milk powder process.

Fluid Milk and the Crumb Process

The major use of fresh, fluid whole milk in chocolate is in sweetened condensed form to make milk chocolate crumb. There are a number of processes for making milk chocolate crumb (Minifie, 1974), all starting with milk, sugar, and chocolate liquor. A general flow diagram for the process of producing milk chocolate crumb is shown in Figure 19.1, and a flow diagram showing the use of the resulting crumb to produce milk chocolate is shown in Figure 19.2. Briefly, the milk and sugar are mixed and moisture is removed through a process similar to that used in the dairy industry to prepare sweetened condensed milk. The chocolate liquor is then mixed in, and additional moisture is removed through a drying process. A typical crumb composition consists of 26% to 35% milk solids, 13% to 18% cocoa solids, and 50% to 65% sucrose. The crumb is generally dried to reach a final moisture content of 0.8% to 1.5% (Haylock and Dodds, 1999).

One major advantage of crumb is its superior keeping quality, attributed to the natural antioxidants in the chocolate liquor portion. Crumb easily can be stored for extended periods of time, as well as shipped to other manufacturing facilities for use in preparation of finished chocolate. The crumb process also offers exceptional flexibility in tailoring the flavor profile of the finished product. The flavor developed during crumb manufacturing uses the Maillard reaction to produce varying degrees of toasted or caramelized flavors. The control of processing time and temperature is very important and results in the generation of key flavor compounds such as furfural, maltol, lactones, and methylketones (Campbell and Pavlacek, 1987). By adjusting the times and temperatures employed, the chocolate maker can adjust the proportions of these components to produce a desired flavor profile.

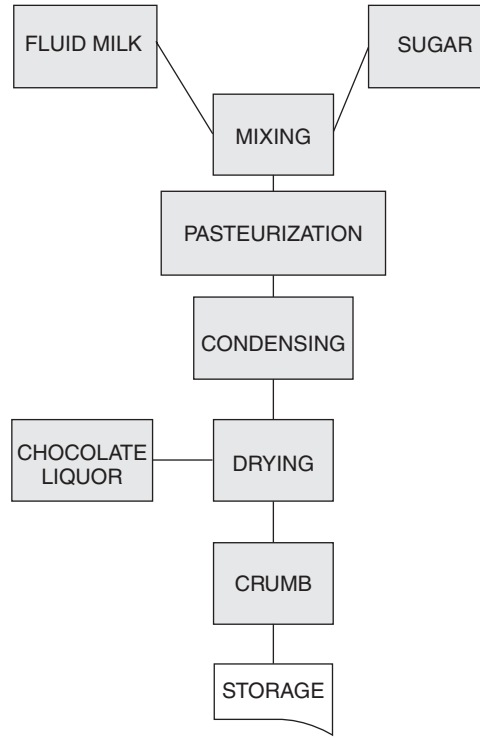

Figure 19.1. Flow diagram for manufacture of milk chocolate crumb.

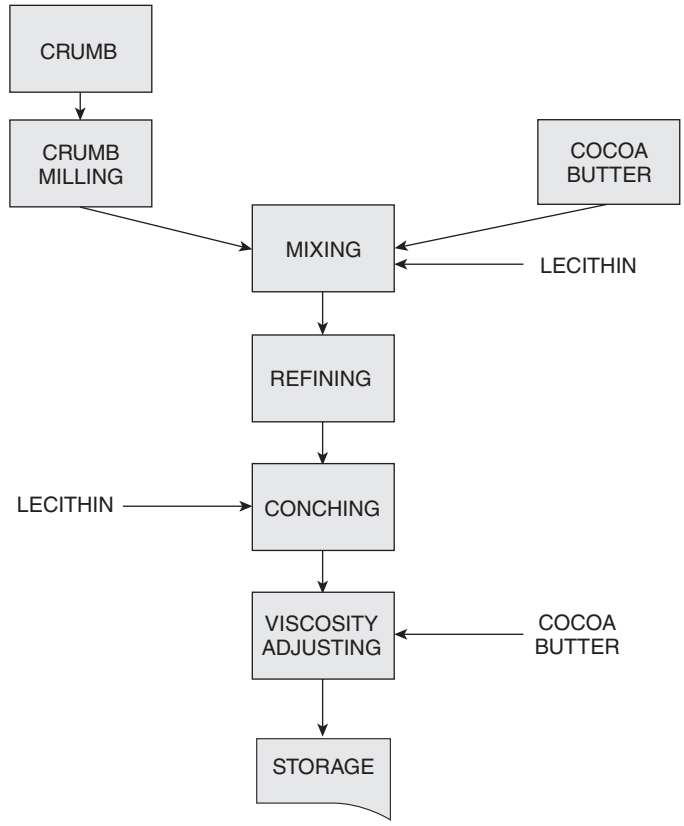

Figure 19.2. Flow diagram for manufacture of crumb-based milk chocolate.

In addition, the crumb process results in a high degree of what is known in the industry as free milk fat. (Campbell and Pavlacek, 1987). Free milk fat refers to milk fat that is not entrapped in the milk solids matrix, and is therefore free to mix with the cocoa butter and cause an increase in the effective continuous phase volume. This increase can cause a reduction in the viscosity of the molten chocolate due to a reduction in the number of particle interactions in the shear flow field.

A scanning electron micrograph of a typical crumb chocolate is shown in Figure 19.3. The image shows two characteristic features of crumb chocolates. First, because many of the sucrose particles present were formed from recrystallization of dissolved sucrose in the milk-condensing process, geometric facets can be clearly observed on the sucrose crystals. Second, milk proteins can be observed associated with the surface of the crystals, rather than being present as discrete particles.

Dry Milk and the Milk Powder Process

The milk powder process can employ either whole milk powder (WMP, full-cream milk powder) or skim milk powder (SMP), or a blend of the two. A general flow diagram for production of milk power chocolate is shown in Figure 19.4. The process flow follows that described earlier, in which milk powder, sugar, cocoa mass, and cocoa butter are mixed, refined, conched, and standardized.

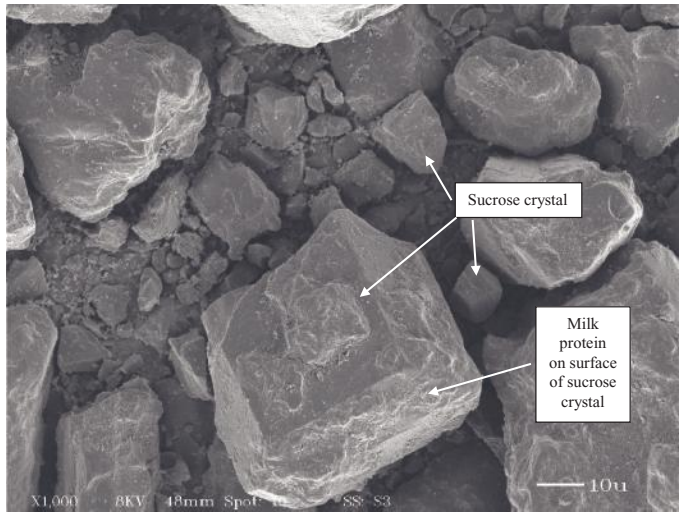

Figure 19.3. SEM image of crumb chocolate showing geometric shapes of the sucrose crystal facets resulting from recrystallization of sucrose during crumb preparation, and the presence of milk proteins associated with the surface of the sucrose crystals.

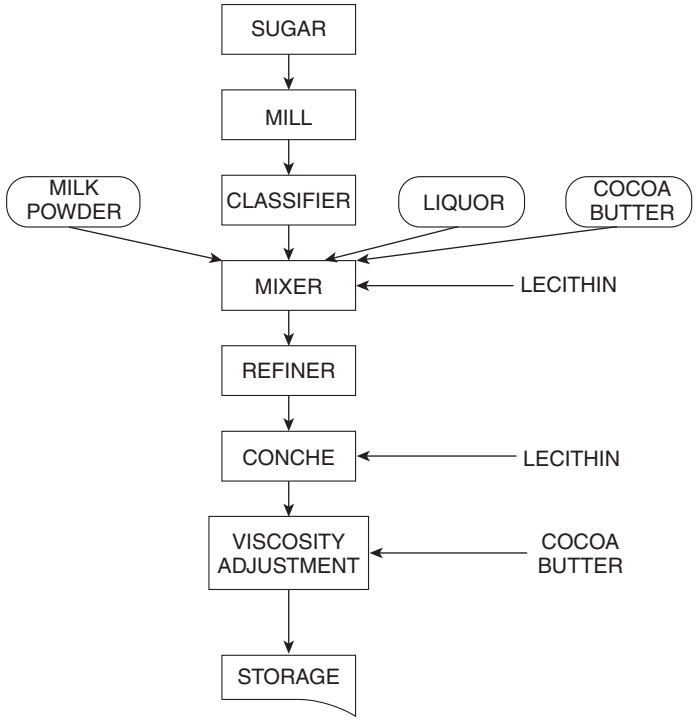

Figure 19.4. Flow diagram of milk-powder-based chocolate manufacture.

Whole Milk Powder

Historically, roller-dried whole milk powder has been the preferred milk powder for chocolate production. Roller-dried whole milk powder is traditionally used in milk chocolate at the level of 12% to 25% by weight of the chocolate (Hansen and Hansen, 1990). Roller drying is accomplished by the direct heating of a thin layer of evaporated milk on a rotating drum, producing a sheet of dry milk that is subsequently powdered in hammer mills. The resulting powder consists of compact particles with very little occluded air and sharp edges as a result of crushing (Cáric and Kalab, 1987).

The main advantage of roller-dried whole milk powders is the high content of free fat which results in favorable rheological properties during mixing and conching, allowing reductions in cocoa butter and energy consumption (Attaie, et al., 2003). The principal disadvantage of roller-dried whole milk powder is that the process is economically unfavorable compared to spray drying, due to lower throughput through the drying process, as well as the requirement for more specialized equipment. These economic implications generally have caused the dairy industry to move toward spray drying as the preferred method of producing milk powder. The authors are aware of several companies in Europe currently supplying roller-dried milk powder, but in the United States and most of the rest of the world, roller-dried milk powder is very difficult and costly to source.

Spray drying is currently the most common method of producing whole milk powder for chocolate manufacture. The powder microstructure is affected by the method of atomization and dryer operation conditions (Cáric and Kalab, 1987), with the strongest influence on the internal porosity of the resulting milk powder particles and the amount of free fat. In spray-dried whole milk powder, amorphous lactose forms a continuous matrix in which proteins, fat globules, and air cells are dispersed (Aguilar and Ziegler, 1993). A modification of typical spray dried WMP that is available to chocolate manufacturers is the so-called high free fat whole milk powder. (Liang and Hartel, 2004). To manufacture this product, the fluid whole milk is separated into skim and cream streams, which are co-spray-dried in a way that the bulk of the milk fat is on the surface of the milk powder particles, thereby offering similar functional benefits of roller-dried milk powder.

The major disadvantage of whole milk powder in chocolate making, regardless of whether it is roller dried or spray dried, is its relatively short shelf life. A recent study showed that WMP lost fresh flavors and developed grassy off flavors by three months of age (Lloyd, et al., 2009). By six months of age, the same milk powder exhibited painty off flavors, which could subsequently be detected in chocolate made with the milk powder. The development of painty off-flavors can be delayed by packing the WMP in bags with an oxygen barrier layer and nitrogen-flushing the headspace at the time of packaging. Nevertheless, sourcing and maintaining working stocks of good quality WMP can be a challenge for chocolate makers, particularly in parts of the world in which nitrogen-flushing and high oxygen-barrier packaging of WMP are not common practices.

Skim Milk Powder

Spray-dried skim milk powder is generally thought to produce chocolates with a less well rounded dairy flavor profile when compared to chocolates produced with crumb or WMP (Campbell and Pavlacek, 1987). Because SMP has a fat content of less than 1%, it is less sensitive to development of off flavors than WMP, and therefore has a substantially longer shelf life. This longer shelf life, in combination with widespread availability in most parts of the world and rela-

tively low cost, make SMP an attractive dairy source for many chocolate makers. SMP is generally available in low-, medium-, and high-heat varieties; these designations refer primarily to the heat treatment that the milk is subjected to prior to and during drying (Anon., 2003). The most widely available and most commonly used SMP is the low-heat variety, although SMP produced using higher heat treatments can possess some of the cooked and caramelized flavors that are desirable in crumb chocolates. Therefore, the SMP heat treatment can have an influence on the final flavor profile of the chocolate.

Caramelized milk powder also is commercially available. In this product, the fluid milk is combined with sucrose, heated for an extended period of time to develop the caramelized flavors and brown color, and then dried and packaged. The use of these milk powders in chocolate can impart some of the caramelized notes normally found in crumb chocolates.

A scanning electron micrograph of a typical milk powder chocolate is shown in Figure 19.5. The image shows milk solids present as roughly spherical particles, rather than diffuse structures associated with the surface of sucrose crystals as was observed for crumb chocolates. The sucrose particles also show irregular fracture edges resulting from breaking during the milling operation instead of the regular geometric crystal facets observed in the crumb chocolate.

Anhydrous Milk Fat

In milk chocolates made using SMP as the principal dairy source, additional milk fat must be added to meet the local standard of identity and to prevent the milk chocolate from becoming excessively hard. Milk fat sources such as dried cream are used on a very limited basis in the chocolate industry, but in general, the preferred source of milk fat is anhydrous milk fat (AMF). AMF is widely available in many parts of the world, and if handled properly (e.g., stored in cool conditions, protected from light and oxygen), has a shelf life of 12 months or more. In addition, AMF has the advantage of existing entirely as free fat in chocolate,

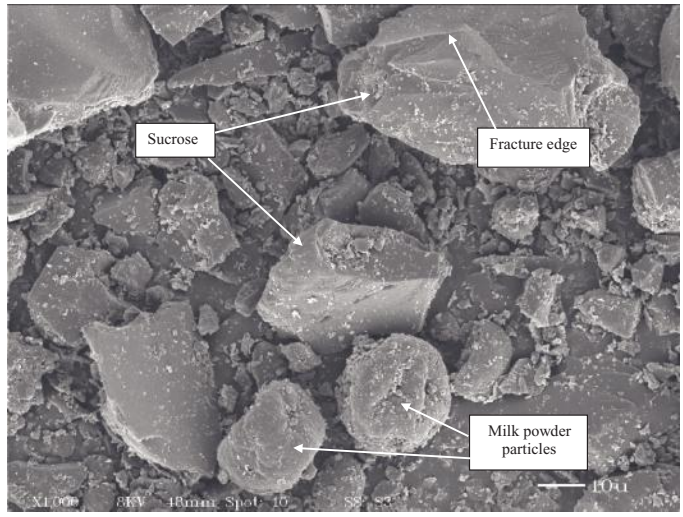

Figure 19.5. SEM image of milk powder chocolate showing milk present as discrete particles and sucrose particles exhibiting fracture edges resulting from the milling process.

thereby producing reduced viscosity and yield stress when compared to an equivalent formula in which some of the milk fat is not free.

The addition of AMF also softens the texture of chocolates, so the amount of AMF can be varied to adjust a chocolate to a desired hardness. AMF also prevents the formation of cocoa butter bloom, but the increased softness of the product can compromise shelf stability of chocolates sold in warm climates. Dark chocolates often contain 1% to 3% of AMF to both soften the texture and to prevent fat bloom during the shelf life of the product.

While chocolate prepared using SMP and AMF has advantages over a WMP chocolate with respect to ease and cost of sourcing ingredients, as well as process viscosity, the choice of milk powder and milk fat system also can have an influence on the flavor and texture of the finished product. Historically, SMP/AMF chocolates were regarded as inferior to WMP chocolates. However, with improvements in process and ingredient technologies in recent years, a number of high-quality SMP/AMF chocolates can be found on the market in many parts of the world. A good-quality product can be delivered using either WMP or SMP/AMF; the chocolate manufacturer must match available supply chain capabilities and economics with the flavor and texture preferences of consumers in the market.

Whey and Other Dairy Ingredients

Recent developments in separation, dairy, and drying technologies have allowed the manufacture of an array of interesting dairy-based ingredients for confectionery, chocolate, and compound coating production. Because whey proteins have functional value as well as nutritional value, whey has been the main source of protein for the development of novel dairy ingredients. Minerals from whey can be reduced by electrodialysis and ion exchange. Whey proteins can be concentrated using ultrafiltration, thereby increasing the protein content from about 0.8% in liquid whey to approximately 80% in a whey protein concentrate. To provide greater nutrition and functionality as well as more than 90% protein, chromatographic techniques in addition to ultrafiltration can be used to produce whey protein isolates. In the formulation of milk and white chocolates, several dairy functional ingredients may be used, either individually or in combination:

- 50% to 90% reduced minerals whey
- Partially delactosed whey
- 35% to 80% protein whey protein concentrates

The protein content of such ingredients is important because it is a key component in the Maillard reaction between amino acids and reducing sugars. This reaction is well known in caramel and toffee manufacturing, although it also takes place to some extent during the conching of milk chocolate.

The level of milk-solids-not-fat (MSNF) in milk chocolate generally varies from 10% to 25%, although a lower limit of 14% applies in most European countries and 12% in the United States. According to European, Canadian, and Codex standards, functional dairy ingredients may be used to formulate milk chocolate in addition to milk and at a level not higher than 5% of total chocolate mass (Anon., 1997; Anon. 1993;, Anon., 1976). In the United States the FDA's standard of identity permits the use of these whey-based ingredients up to 5% only in white chocolate (Anon., 2004).

The practice of including whey and whey derivative ingredients in chocolate can function to develop specific or signature flavors, optimize manufacturing cost structure while maintaining high quality to take advantage of the excellent functional and nutritional characteristics of whey-based products, and provide creamy-milky flavor notes to the finished product. In general terms, replacing

milk solids with whey-based ingredients at a 5% level can enable savings of as much as 8% to 14% of milk powder costs while maintaining a high-quality end product. Furthermore, the addition of functional whey-based ingredients to a milk chocolate formulation can increase the rate of the Maillard reaction and enable the production of caramelized flavor notes in a milk-powder-based chocolate.

In most chocolate products, whey-based ingredients with low mineral content are preferred to avoid a salty flavor which can often be detected in the chocolate. The undesirable salty tasting mineral ions such as sodium, potassium, and chloride should be removed while retaining a high amount of calcium. This extra processing step may be unnecessary if, for example, the chocolate will be used to make a bar with salted nut inclusions.

Milk chocolate is consumed in a variety of ways, as a snack item as the common chocolate bar or as a topping or coating on products such as candy bars, biscuits, cakes, etc. Such diverse application of a single product means that different milk chocolate formulations may be developed for each application to provide both the functional and sensory properties that the manufacturer and consumer expect (Haylock, 1995).

Lactose also can be used to control the sweetness of the finished product. In some regions of the world, it is desirable to produce a chocolate with reduced sweetness. Substituting up to 10% of the sucrose with lactose can help achieve this goal.

Compound Coatings

Chocolate-flavored compound coatings provide an alternative to standard-of-identity chocolate through the substitution of vegetable fats for the cocoa butter. Typical formulas for milk chocolate-flavored and white-chocolate flavored compounds are shown in Table 19.2. Compared to chocolate, the resulting products can be less expensive and often require less complex manufacturing procedures. The organoleptic properties of compound coatings often do not match those of standard-of-identity chocolate, but they allow more flexibility in terms of new and novel textures and applications, and can still provide a very high-quality eating experience. Compound coatings are formulated for use in many applications in which the functionality needed in the fat phase prevents the use of cocoa butter, or when the cost of the finished product becomes an important constraint.

Procedures for manufacture of compound coatings are analogous to those for chocolate manufacture, with particle size reduction generally achieved through the use of media mills. Special attention should be paid to the compatibility of vegetable fats with cocoa butter. Fat incompatibility leads to a softening of the coating due to an eutectic effect that can lead to bloom formation and can influence oil diffusion and shelf life.

Ingredients used in compound coating formulations generally include sugar, chocolate

Table 19.2. Typical compound coating formulas.

	Chocolate-flavored compound		White compound	
	Tempering	Non-tempering	Tempering	Non-tempering
Sugar	48.10	48.10	52.55	52.55
CBE	30.00	—	30.00	—
CBS	—	30.00	—	30.00
Demineralized whey	5.00	5.00	5.00	5.00
SMP	9.00	9.00	12.00	12.00
Cocoa powder	7.50	7.50	—	—
Lecithin	0.30	0.30	0.30	0.30
PGPR	0.05	0.05	0.05	0.05
Vanillin	0.05	0.05	0.10	0.10

liquor (mass) and/or cocoa powder, vegetable fat, dried dairy ingredients, and surfactants, generally lecithin and polyglycerol polyricinoleate (PGPR) in combination. Coatings are typically made with particle size from 15 to 50 micrometers. As with chocolate, particle size affects the coating texture. A coarse product may be less expensive to produce, but the coating may taste gritty if too coarse.

The fat component of a coating has a strong influence on the sensory characteristics of the finished product, particularly with respect to melting behavior in the mouth. The melting point of cocoa butter in a well-tempered chocolate ranges from approximately 26°C to 33°C. Fats in compound coatings are typically customized to a 32°C to 37°C melting point. However, there are a wide variety of fats and oils that can be used to tailor the melting point of a compound coating to achieve many finished product properties (Weyland, 1998).

Some compound coatings may be tempered, but many compound coatings are currently formulated with non-tempering fats known as cocoa butter substitutes (CBS). Cocoa butter substitutes are lauric fats and are totally incompatible with cocoa butter; therefore, the amount of cocoa butter in the formula coming from chocolate liquor or cocoa powder should be kept to a minimum, generally 5% of the formula weight or less. Use of higher levels can lead to an unstable product prone to bloom formation. If a tempering fat is desirable, cocoa butter equivalents (CBE) are the vegetable fat of choice. CBE are fats that have the same triglyceride structure as cocoa butter. Therefore, both fats could be mixed in any proportion. On average, good-quality CBEs tend to be 10% to 15% less expensive than cocoa butter. A third alternative is the use of cocoa butter replacers (CBR). These fats are less compatible with cocoa butter than CBE, but more so than CBS. The proportion of CBR to cocoa butter is often in the range of 1 : 4, depending on the desired attributes of the finished product and the quality of the CBR.

Table 19.3. Sample nougat formulation.

Part I: Frappe—26.50%	% w/w of finished product
Egg white solids	21.50
Partially denatured WPI-90	11.35
Sucrose, 6 ×	40.10
Water	26.55
Salt	0.50
Part II: Syrup (loss from boiling 12%–13%)–50%	
High-fructose corn syrup	36.00
Sucrose, granulated	45.00
Water	19.00
Part III: Additional ingredients–23.50%	
Sucrose, 6 ×	74.85
Cocoa powder, 10%–12% fat	13.80
WPC-34	2.50
Vegetable fat	8.00
Flavor	0.85

Dairy Ingredients in Compound Coating Formulations

Because compound coatings do not have standards of identity, product developers have much more flexibility in the use of whey-based ingredients than in chocolate formulas. If milk powder is used to prepare a coating, it is generally SMP. Because the melting point of the coating fats can be tailored, there is no need to use AMF to adjust the hardness of the finished product, and therefore it is seldom used. Whey-protein-based ingredients are often used as milk solids sources in the formulation of milk-chocolate-flavored coatings for ice cream, candy bars, and other enrobing applications, replacing milk powder in part or entirely.

Sweet whey, reduced-minerals whey, and whey protein concentrates and blends are used as a total or partial replacement for milk powder in coating formulations. Table 19.3 shows potential formulations for milk chocolate and white compound coatings for different applications.

Sugar Confectionery

Boiled sugar confectionery products are made by cooking mixtures of sugar, glucose

syrup, water, and other ingredients. It is the combination of the multiple ingredients and processing options that allows the confectioner to manipulate chemical and physical interactions to produce a wide range of products (Jewell, 1986).

The range of textures that can be achieved in sugar confectionery is diverse, spanning from hard (high boils), to soft (nougats, marshmallow), to chewy (caramel). The major ingredients that contribute to these characteristics are sugars (including sucrose and invert and glucose syrups), fats, and proteins. Dairy ingredients play a critical role in the texture, flavor, and manufacturing of many sugar confectionery products.

Caramel and Toffee

Both caramel and toffee have the same ingredients; the difference between the two is that the final moisture content is less in toffee than in caramel. Toffee has 3% to 6% final moisture and is generally darker in color, whereas caramel has 6% to 12% moisture and is lighter in color (Warnecke, 1996; Guelfi, 1988; Brown, 1993).

Caramel is one of the most versatile and widely used confectionery products. Caramel alone can be a finished product, it can be used as a confectionery center in an enrobed or molded piece, or it can be used as a component in combination with other ingredients such as cookies, pretzels, nougat, or marshmallow.

Caramel is produced by blending water, glucose syrup, refined or brown sugar, emulsifier, milk fat or vegetable fat, and dairy solids. The mix is concentrated to a high solids content by cooking to approximately 116°C. This process helps to develop the characteristic color and flavor through caramelization and the Maillard reaction (Biondi, et. al., 1993; Anon., 1997).

Several ingredient factors influence the Maillard reaction, and thus the flavor, color, and texture of caramels. First, the amount and type of reducing sugars contribute differently to the browning reaction; monosaccharides are more reactive than disaccharides. The composition of the dairy components in a caramel formulation influences the browning reaction due to varying amounts of lactose. Sucrose is the main bulking and sweetening agent in caramel; it reduces stickiness and cold flow. However, the higher the amount of sucrose in the formula, the greater the likelihood of crystallization during shelf life. Glucose syrup contributes to body, texture, chewiness, and sweetness of caramel. Generally, higher levels of polysaccharides in the syrup produce firmer and chewier caramels.

Second, the composition of the protein can play a role in the caramelization process. Caramels can be formulated using various dairy ingredients. It is important to balance the formulation, taking into consideration the type and amount of protein present. Whey protein concentrates offer a cost-effective alternative to formulate caramels with good eating quality and excellent processability. However, in certain applications such as stand-up caramels, care should be exercised when selecting the whey ingredient because a minimum amount of casein is needed for the appropriate structure. In general, the higher the level of dairy solids in caramel, the better the flavor, color, and stand-up qualities.

Third, the fat-containing ingredients used in the formulation of caramels influence the texture, mouth feel, and shelf life of the finished product. Fats are used as flavor carriers, to reduce stickiness, and improve machinability and stand-up quality. While the amount of fat can vary from 5% to 20% of the formulation, 10% to 12% fat is typical. Fats provided from dairy sources and vegetable fats such as coconut oil are common, and should be properly emulsified to prevent migration to the surface, which can cause greasy mouth feel and development of rancidity. If there is an adequate level of dairy protein present, there may be enough natural emulsification present. Shear agitation or

homogenization also helps provide well-dispersed small fat globules. Emulsifiers such as lecithin (less than 1%) or mono- and diglycerides (5% to 10% of the fat weight) aid in producing a stable emulsion. In addition to emulsification, mono- and diglycerides contribute to machinability, stand-up, and hardness of the finished caramel product.

Traditionally, caramel has been made using sweetened condensed milk as the principal dairy ingredient. Because sweetened condensed milk is costly to ship and has a relatively short shelf life due to its water content, many caramel producers find supply chain efficiencies by procuring SMP and preparing a condensed milk intermediate on site by reconstituting the milk powder.

It is advisable that powdered dairy ingredients be reconstituted with warm water (50°C to 60°C), preferably in an homogenizer prior to addition to the cooking vessel. Pre-blending of the dairy powder with some of the formulation sugar also prevents lumping during the recombination process. If no homogenizer is available, the dairy ingredients can be reconstituted in the following manner. Place a pre-determined amount of warm water heated to 72°C in a kettle. Slowly add the blended dairy powders with enough agitation to give the solution a creamy consistency. Mix for at least 15 to 20 minutes. If the recombined dairy blend shows signs of curdling, it is advisable to add a neutralizing agent, usually disodium phosphate at a level of 0.01% to 0.05% of the total protein.

High-quality caramels can be produced using sweet whey and whey protein concentrates as partial or total replacements of the milk component; however, care must be taken not to exceed the saturation point of the lactose. In formulations with high levels of whey components, the high level of lactose can result in a system that is supersaturated in lactose, and the formation of lactose crystals can cause organoleptic defects over the shelf life of the confection. In these types of formulas, commercially available lactose-hydrolyzed whey preparations can be successfully used to produce a shelf-stable product.

Aerated Confectionery

An aerated confectionery product is a dispersion of a gas in an aqueous phase that is a highly concentrated syrup made up from a variety of sugars and whipping agents. The density of the finished product is reduced by the presence of gas, usually air. Nitrogen can be used when a high amount of fat is present (Jackson, 1990; Anon., 1994). Aerated confectionery is produced by vigorous agitation of the syrup. Stability is obtained by the addition of a whipping agent or stabilizer.

At the simplest level the air is incorporated by beating at various speeds in an open pan. Beating creates a series of cavities which entrap air in the form of large bubbles. While continuing beating, the largest air bubbles are broken into smaller units. The viscosity of the final mix increases depending on the amount of air incorporated in the product.

Stability of the aerated confection is obtained by the presence of high-molecular-weight foaming agents, which create a protein network within the foam to help stabilize the final structure. That protein semi-rigid network is present in the syrup or continuous phase of the confectionery which surrounds each of the air cells (Jackson, 1990; Anon., 1994). These protein foaming agents carry both hydrophobic and hydrophilic groups within their structure. The hydrophobic group is absorbed on the surface layer of the bubble, while the hydrophilic groups are directed to the aqueous phase. In the next stage the protein chains may begin to untangle and lie along the surface film. This helps to hold the air cells in a rigid and stable network.

Common aerating proteins used in the formulation of aerated confectionery include gelatin, soy, and modified dairy proteins. Within the last category, both partially denatured WPCs (80% protein) and partially

denatured WPIs (more than 90% protein) offer a cost-effective alternative to the confectionery manufacturer when used in combination with the other aerating agents.

Nougat is a common aerated confection that can be formulated using whey-based ingredients. It is an aerated high boiled syrup containing fat that has been stabilized by the addition of a whipping agent. A basic nougat formulation is shown in Table 19.3. The formula and process used to produce nougat can be adjusted to give a range of textures, varying between a long-eating, chewy, non-grained product and a short-eating, soft, fine-grained product.

The texture of nougat is influenced by the ratio of sugar to glucose syrup to invert sugar syrup, the final moisture content of the nougat, the ratio of the liquid phase to the solid phase, the type of whipping agent, the degree of aeration, and the quantity and type of added ingredients, e.g. fat, nuts, cherries, etc.

Nougat can be produced using either batch or continuous methods. The batch process consists of boiling water, sugar, and corn syrup under vacuum to a moisture content of about 8% at a temperature of 120°C. A vacuum cooker is used not only to reduce the time of boiling but also to produce a cooked syrup at a lower temperature—the higher the temperature, the longer the beating time. The vacuum-cooked syrup is transferred to a robust and powerful atmospheric whipping machine that can operate at two speeds, low for mixing and blending and high for aerating. A protein-based whipping solution is blended into the cooked mass before being aerated at high speed, when the density is reduced to 0.6 g/ml. A small quantity of icing sugar to seed crystallization is blended into the aerated product at low speed.

Vacuum-aerated products are another common type of aerated confectionery product which can be formulated using whey-based ingredients, for example, malted milk balls (Wolfe, 1995). The ingredients used in the formulation mainly consist of corn syrup, sugar, protein, flavor, and color. The quality of the ingredients is critical to the formation of the air bubbles.

Whey powders, either sweet whey or partially demineralized whey, can be used as a cost-effective protein source in the formulation of this type of confectionery. A minimum of 10% protein in the whey is critical to avoid product texture collapse after vacuum processing. Many of these products are tolerant of some salty flavors, so extensive demineralization often is not required.

For the production of vacuum-aerated confections, batching of ingredients is fairly straightforward. Corn syrup and sugar are cooked to the desired solids (approximately 90%) and transferred to a suitable mixer where the dry ingredients were previously added. A thorough mixing process to allow for a uniform mass then occurs. The mass is unloaded into a sheeting device and the sheet conveyed to the forming rolls.

The centers are then stored until ready for expansion. Prior to expansion, the centers must be uniformly heated to a desired temperature of about 80°C to obtain the desired amount of expansion. The preheated centers are then conveyed through a vacuum tube where vacuum is applied. Approximately 1% moisture is removed and the product is cooled sufficiently so that the center will not collapse. Excessive residual moisture causes the extremely hygroscopic centers to become wet and sticky, eventually collapsing the cell structure completely. During the panning process the centers should be completely covered with chocolate or compound coating to prevent moisture pickup (Wolfe, 1995).

Conclusion

Milk and dairy ingredients play an important role in the confectionery industry. These ingredients provide characteristic textures and flavor profiles in chocolate and confectionery products, and permit the products

to conform to relevant local standards of identity. Selection of the appropriate dairy ingredients can aid the confectionery manufacturer in producing a high-quality, economically viable product with high consumer acceptance.

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Chapter 20

Dairy Ingredients in Infant and Adult Nutrition Products

Jeffrey Baxter, Steven Dimler, and Nagendra Rangavajala

Introduction

A wide variety of nutritional products, ranging from infant feeding to critical care nutrition, are generated using bovine milk-derived materials as the principal source of high-quality protein and nutrients. Nutritionally, milk is considered to be a nearly perfect food because it provides more essential nutrients in significant amounts than any other single food. Milk is an excellent source of calcium and phosphorus for bones and teeth, and it contains several fat- and water-soluble vitamins in significant amounts.

This chapter discusses the use of dairy ingredients and technologies in the development and commercialization of infant, medical, and sports nutritional products, along with benefits these products confer to the health needs. A commentary on future research trends and opportunities is presented.

Infant Nutrition

Any discussion of infant formula development is stimulated by an ever-deepening understanding of the composition of breast milk. The ability of the industry to mimic the composition (or at least the physiologic response) using commercial formulas is driven by such knowledge, and by the increasing sophistication of commercially available ingredients. Today there is a growing assortment of general and specialized infant formulas. One of the greatest achievements of nutrition research in the past several decades has been the introduction of specialty formulas used to feed premature and very low-birth weight infants.

Although the compositions of the available infant formulas approach mother's milk, they are subjected to strict regulations to safeguard the safety of the infants. These regulations, coupled with safety and shelf stability requirements, currently limit the ability to completely replicate the composition of human milk. For example, the ability to include bioactive factors such as active enzymes, immunoglobulins, and growth factors that are present in human milk in ready-to-feed versions of these formulas is technologically limited due to heat processing to which liquid formulas are subjected and that result in denaturation that affects the functionality of these bioactive factors. Furthermore, when commercial sterility is achieved, the biologically functional 3-D structures of many of these factors are disrupted, and activity is reduced or eliminated. Another aspect of mother's milk is its changing nature through lactation. While some manufacturers have produced staged formulas, this still does not quite mimic the naturally delivered nutrition.

The basic regulations for infant nutrition are the U.S. Infant Formula Act, the European Food Safety Authority (EFSA) regulations,

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and the Codex Alimentarius. In addition, individual countries may have their own regulations and societies with varying interpretations of the nutritional needs of infants. Important societies (which also issue recommendations) include the American Academy of Pediatrics (AAP), European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), and Life Science Research Office (LSRO). The main points of the Codex Alimentarius are shown in Table 20.1.

Several countries also have additional regulations governing infant formula composition and marketing. As the industry endeavors to bring formulas closer to human milk

Table 20.1. Main points about Codex Alimentarius standards for Annex 1 (infant formula, IF) and Annex 2 (follow On formula, FOF).

		IF	IF	FOF	FOF
		Minimum	Maximum	Minimum	Maximum
Energy	kJ/100 ml	N.S.	N.S.	250	355
	Kcal/100 ml	N.S.	N.S.	60	85
Protein	g/100 kJ	0.43	0.96	0.7	1.3
	g/100 kcal	1.8	4	3	5.5
Choline	mg/100 kJ	1.7	N.S.	N.S.	N.S.
	mg/100 kcal	7	N.S.	N.S.	N.S.
Lipids	g/100 kJ	0.8	1.5	0.7	1.4
	g/100 kcal	3.3	6	3	6
Carbohydrates		N.S.	N.S.	N.S.	N.S.
Minerals					
Na	mg/100 kJ	5	14	5	21
K	mg/100 kJ	20	50	20	N.S.
Cl	mg/100 kJ	14	35	14	N.S.
Ca	mg/100 kJ	12	33	22	N.S.
P	mg/100 kJ	6	N.S.	14	N.S.
Mg	mg/100 kJ	1.4	N.S.	1.4	N.S.
Fe	mg/100 kJ	0.04	N.S.	0.25	0.5
Zn	mg/100 kJ	0.12	N.S.	0.12	N.S.
Cu	µg/100 kJ	14	N.S.	N.S.	N.S.
I	µg/100 kJ	1.2	N.S.	1,2	N.S.
Se	µg/100 kJ	N.S.	N.S.	N.S.	N.S.
Mn	µg/100 kJ	1.2	N.S.	N.S.	N.S.
F	µg/100 kJ	N.S.	N.S.	N.S.	N.S.
Vitamins					
Vitamin A	IU/100 kJ	60	120	60	180
Vitamin D	mg/100 kJ	10	25	10	30
Vitamin C	µg/100 kJ	1.9	N.S	1.9	N.S.
Vitamin B_1	µg/100 kJ	10	N.S	10	N.S.
Vitamin B_2	µg/100 kJ	14	N.S	14	N.S.
Vitamin B_3	µg/100 kJ	60	N.S	60	N.S.
Vitamin B_5	µg/100 kJ	9	N.S	11	N.S.
Vitamin B_6	µg/100 kJ	1	N.S	1	N.S.
Vitamin B_{11}	µg/100 kJ	70	N.S	70	N.S.
Vitamin B_{12}	µg/100 kJ	0.04	N.S	0.04	N.S.
Vitamin K	µg/100 kJ	1	N.S	1	N.S.
Vitamin H	µg/100 kJ	0.4	N.S	0.4	N.S.
Vitamin E	IU/100 kJ	0.15	N.S	0.15	N.S.

N.S., not specified

Formulas with a higher protein content than 1.8/100 kcal should contain a minimum of 15 µg vitamin B_6 per g/protein

Isolated amino acids may be added to infant formula only to improve its nutritional value for infants. Essential amino acids may be added to improve protein quality, but only in amounts necessary for that purpose. Only natural L forms of amino acids shall be used

The Ca : P ratio shall not be less than 1.2 and not more than 2.0

functionality, a key challenge the industry faces is delivering innovation within regulatory, cost, and benefit expectations.

Innovations in Infant Nutrition

The first intentionally designed infant formulas were produced in response to problems generated by the use of condensed skim milk as a mother's milk replacement in the late 19th and early 20th centuries. Differences in composition between bovine and human milks (Table 20.2) and vitamin and mineral needs resulted in significant nutritional deficiencies and unacceptably high infant mortality. Infant formula innovations across the centuries are given in Table 20.3 (Hansen and Diener, 1997; Benson and Masor, 1994; Committee on the Evaluation of the Addition of Ingredients New to Infant Formula, 2004).

As shown in Table 20.4, the milk protein combinations commonly used in commercial infant formulas, while adequate in supplying protein nutrition, still do not replicate the protein composition of breast milk. The more esoteric and complex benefits derived from

Table 20.2. General composition of human and cow's milk.

Contents	Human milk	Cow's milk
Fat		
Total (g/100 ml)	4.2	3.8
Fatty acids <8°C*	Trace	6
Polyunsaturated fatty acids (%)	14	3
Protein (g/100 ml)		
Total	1.0–1.3	3.0–3.3
Casein	0.3	2.5
Whey	0.7	0.5
Casein : whey ratio	40 : 60	80 : 20
Carbohydrate (g/100 ml)		
Lactose	7.0	4.8
Oligosaccharides	0.5	0.005
Minerals (g/100 ml)	0.2	0.7

*Chain length, in carbons
Source: www.unu.edu/unupress/food/8F174e/8F174E04.htm

Table 20.3. Infant formula developmental timeline.

Future	Will depend on understanding of disease states, nutrient bioavailability, etc.
2000s	Long-chain polyunsaturated fatty acids (LCPUFAs) introduced
	Choline fortification
	Probiotics/prebiotics
Late 1990s	Nucleotide fortification
1980s	Taurine fortification
	Infant Formula Act
1960s	Iron fortification considered
	Renal solute load considered
	Concentrated liquid formula
	Whey : casein ratio similar to human milk
1940s	Hypoallergenic formula for allergy management
	Protein content of the formula considered
1920s	Commercially available soy formula
1900s	Carbohydrate additive
	Moores and Ross Milk CO makes Franklin Infant Food—forerunner of Similac
	Human milk fat simulated in SMA formula
1850s	Human milk simulation by adding carbohydrates to cow's milk
1750s	Cow's milk dilution

Table 20.4. Major protein components of human milk and major commercial infant formulas.

Protein (g/liter)	Breast milk	Commercial formulas**
Total protein	Apprx. 6.9–9.2*	14.0
α-$_s$ caseins	0	2.7
β-casein	3–3.4	2.2
κ-caseins	Apprx. 0.5	0.7
β-lactoglobulin	0	3.5
α-lactalbumin	2.7–3.3	1.2
Lactoferrin	1.4–1.9	0
Glyco-macro peptide	0	1.4
Other proteins	1–2	2.3

*Amount of protein decreases over time; excludes non-protein nitrogen
**Milk base enriched with demineralized whey protein, data provided by Paul Johns, Abbott Laboratories.

mother's milk may still be reduced or absent from formula, though that is changing.

Human milk proteins differ from bovine milk in several important ways. Human milk is whey dominant, with approximately 60% of the protein from whey, while cow's milk is casein dominant, with approximately 80% of the protein from casein. Breast milk casein contains mostly β-casein with some κ-casein (glycosylated with sialic acid) and virtually no α-casein. Also in contrast to bovine whey, the human whey fraction contains no β-lactoglobulin and relatively high levels of lactoferrin. Lactoferrin is a multifunctional protein with antimicrobial activity that provides immune functionality as well as significant and biologically functional iron-binding capacity.

Standard commercial formulas usually have a combination of bovine caseins, predominately β-lactoglobulin in the whey protein, and only trace amounts of lactoferrin. While casein-dominant formulas were prevalent 20 to 30 years ago, a more balanced delivery of whey and casein is now the norm. Advances in protein separation technologies resulted in commercial availability of fractions of milk proteins such as bovine β-casein, lactoferrin, and bovine whey protein enriched in α-lactalbumin, which may be used to mimic human milk protein composition. However, their high costs of manufacture along with insufficiently defined clinical benefits have thus far prevented their widespread use in commercial formulas.

Efforts have been made to produce some of the more esoteric proteins (especially lactoferrin) via fermentation of genetically modified microorganism species, but these are not yet commercially viable. Greater research into fractionation of milk and further experience in alternate production avenues will ultimately enable exploration of the potentially unique properties of these proteins. Some examples include β-casein with biological functionality related to its phosphorylation state and κ-casein with bacterial adhesion modification and other functionality, dependent on its glycosylation state. Furthermore, α-lactalbumin has calcium- and zinc-binding properties, and with no free thiols the pure form does not form gels upon denaturation or acidification. β-lactoglobulin forms a unique stable foam and has useful gelation properties. Lactoferrin is potentially bactericidal with structural similarities to the lysozymes. It is evident that the exploitation of these proteins is just beginning.

Byproduct streams from production of major food ingredients from milk are another important source of interesting ingredients. Most notable among these are the byproducts

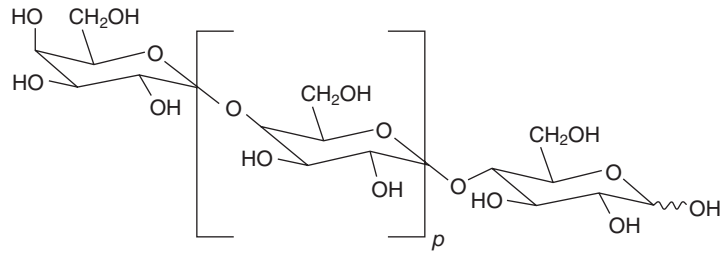

Figure 20.1. Structure of galacto-oligosaccharides (p = 0 – 6).

of acid casein and cheese making, both of which are sources of whey protein products. However, the whey protein materials derived from these two sources are somewhat different because the use of rennet in cheese production actually hydrolyzes a peptide bond of κ-casein, cutting the protein approximately in half, with one half carrying all of the glycosylation of the protein. This heavily glycosylated κ-casein fragment is called glycomacropeptide (GMP), and is a significant component of cheese or sweet whey. The byproduct of acid casein as a whey protein source produces sour or acid whey, which does not contain significant GMP.

Increasing knowledge about factors that affect digestibility, causes of intolerance, and milk-protein allergy has had an enormous impact on the protein composition of infant formulas. Partly or completely hydrolyzed whey and milk proteins are available for these specific purposes. More recent formulas have focused on the benefits of partially hydrolyzed protein systems, promoting their ease of digestion. These partially digested proteins also offer a wide variety of peptides with potential physiological benefits to infants. These improvements affect the cost of formulas, which is offset by the increased benefits to infants.

Milk protein intolerance also can be addressed by use of alternative protein sources, most notably soy protein. Outside the United States, other protein sources, such as rice protein, are also used in commercial formulas designed for intolerant infants, but these have not yet achieved significant market share.

Lactose intolerance in infants has been addressed by use of soy protein formulas or formulas using lactose-free or very-low lactose dairy protein. Lactose intolerance has been associated with fussiness and gassiness; the incidences of true clinical lactose intolerance may not be as widespread as thought.

Another breakthrough development in recent years has been the introduction of galacto-oligosaccarides (GOS) or transgalactosylated oligosaccharides (TOS) in infant and toddler products (Figure 20.1). Human milk contains a variety of oligosaccharides, with associated physiological benefits. A review of the wide variety of structures and their putative functionalities is beyond the scope of this chapter; however, a few recent reviews are listed at the end of the chapter for further information. The functionality of such oligosaccharides can be partially mimicked in infant formula by addition of GOS to help stimulate positive bacterial growth in the digestive tract, thus, they are termed prebiotics.

Measurable lactulose levels can be found in formulas, especially in retorted liquid infant formulas that contain lactose. Lactulose may help in softening the infants' stools. However, excessive lactulose can create intestinal disorders, and levels of this compound are controlled by a combination of formulation and processing.

Combinations of vegetable and tropical oils have been used to approximate breast milk fat. Butter fat (relatively high in saturated fat and cholesterol) is not a good approximation of the lipids in breast milk; they are high in shorter chain unsaturated fats and essential oils, with moderate levels of cholesterol and saturated fats. Research continues into the potential physiological benefits of the phospholipids in bovine milk fat. Interesterification of oils allows for the more appropriate placement of the palmitic fatty acid on the C-2 position, which has substantial impact on digestion and absorption routes. More recently, long-chain polyunsaturated fatty acids (docosahexaenoic acid and arachadonic acid) are routinely added to infant formulas, because studies indicate a positive effect on brain development as well as other health benefits.

Probiotics are defined by the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) as "Live microorganisms, which when administered in adequate amounts, confer a health benefit on the host," and their use is gaining great interest. Milk and whey proteins offer good encapsulation properties that could allow for delayed release of these probiotics. Further work is in progress to develop encapsulation technologies to allow introduction of live microorganisms into ready-to-use liquid formulations. Alternatively, interesting packaging developments allow mixing of the probiotics with the liquid nutritional product at use, but this currently adds significantly to packaging costs. Introduction of these live bacteria into liquid formulas is still very technically challenging, but can be achieved via dry blending into powder products. Development of technologies in the near future is anticipated, which would allow use even in liquids.

Major infant formula manufacturers offer products for infants with various metabolic disorders such as significant prematurity and low birth weight (and even very low birth weight) as well as other neonatal intensive care unit (NICU) products. These are available in different caloric density and nutrient distribution forms and a variety of convenient packages. These product innovations have enabled better outcomes for infants in the NICU environment. The metabolic disorder formulas include those for inborn errors of amino acid metabolism, which deserve special mention because of the unique protein source, free purified amino acids. Their use also presents some limitations on acceptable carbohydrate sources, because high reducing sugar content can result in significant Maillard browning. These reactions can generate product acceptability issues (flavor, color, and odor) as well as potential clinical impacts. Such considerations also affect formulas with heavily hydrolyzed protein systems, which have similar reactivity. Finally, free amino acids are absorbed by the body in a different fashion than peptides, which increases the total protein requirements for optimal growth. Thus, a completely free amino acid (or elemental) formula is only used when physiologically demanded.

Comments on Manufacturing

Commercial infant formulas are primarily available as ready-to-feed or concentrated liquids and powders (Figure 20.2). Manufacturing of infant formulas is based on either complete integral processes or a base mix preparation, consisting of main components combined with a blending operation to specialize the recipes for target groups. The manufacturing process typically consists of reconstitution of ingredients (such as milk or milk solids, and whey protein materials, lactose or maltodextrins, corn syrup or other carbohydrate sources, minerals, lipids and fats, emulsifiers and stabilizers, and vitamins and trace nutrients) in a specific sequence to enable heat and shelf stability, followed by

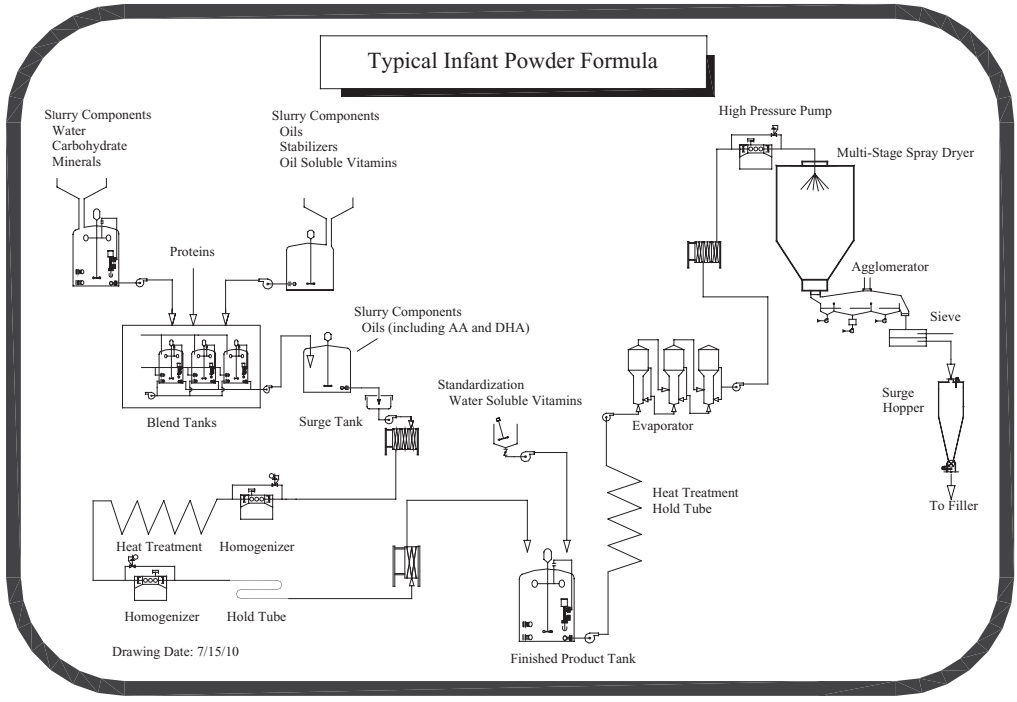

Figure 20.2. General manufacturing scheme for a typical infant formula.

heat treatment and homogenization. Liquid products are heat sterilized and aseptcially dispensed into hermetically sealed containers or containers that are sealed and then heat treated (called retort processing). Powdered products are spray dried and dispensed into containers that are typically flushed with nitrogen gas to reduce head space oxygen levels and stabilize oxygen-labile ingredients (e.g., certain vitamins, unsaturated fats).

Certain formulas, particularly those containing extensively hydrolyzed protein or free amino acid protein systems, require additional stabilizers and emulsifiers to provide emulsion stability during processing and shelf storage, because this is usually a function of the intact or relatively intact proteins. Prior to commercially distributing the products, each batch is sampled and tested to ensure that nutritional and quality specifications are met. Quality control procedures are rigorous with regard to microbiological quality and nutritional profiles to ensure safety and adherence to applicable laws, which are enforced in virtually every country. Typically, some specific nutrient requirements differ slightly between countries, challenging the development of single, globally acceptable products.

Adult Nutrition

A wide variety of nutritional products use milk-derived ingredients as a major source of nutritional content. Adult nutrition products can be classed into three broad categories:

1. Medical/therapeutic nutrition products: Provide nutritional intervention in patients who are malnourished, have

health conditions such as renal disease or diabetes, or have special dietary needs such as those in critical care.

2. Performance nutrition products: Provide support to recover and/or enhance performance in sports or exercise endurance.
3. Healthy living products: Help manage health proactively to prevent diseases later in life. Examples include products designed for maintaining healthy blood lipids and immune benefit; these may be meal enhancers or meal replacements.

Nutritional products are available for children with normal or challenged digestive systems, generally healthy adults, physically active sports enthusiasts, adults with diseases that require specific nutritional formulas (particularly the elderly), and for many others.

Typically, products for adult applications use protein systems with combinations of casein or milk, soy, and demineralized whey proteins. The combinations are adjusted to optimize flavor, nutrition, physical appearance, and cost. As the total protein level increases, partially hydrolyzed proteins are used for viscosity reduction. The degree of hydrolysis is often limited by flavor constraints. Other proteins (in particular wheat gluten and its hydrolysates) also can be introduced when special needs dictate nutrition enriched with a particular amino acid (e.g., glutamine or arginine) or combinations of amino acids (e.g., branched-chain amino acids). The best known among these are diets enriched in glutamine or arginine for special use in critical care situations and protein supplements with high levels of branched-chain amino acids (leucine, isoleucine, and valine) for sports protein supplements.

Reduction of lactose is commonly preferred for the adult population due to some degree of lactose intolerance. The level of acceptable lactose dictates whether caseinates, milk protein isolates, or milk protein concentrates are used. One example of a reduced calorie carbohydrate is D-tagatose, which is manufactured from lactose. It has prebiotic effects and is suitable for diabetics. The choice of alternate carbohydrates includes various maltodextrins to help control osmolality and sucrose to increase flavor acceptability and reduce non-enzymatic browning, which can be particularly prevalent in products with extensively hydrolyzed proteins or addition of free amino acids, as previously indicated. Prebiotic carbohydrates such as galacto-oligosaccharides or D-tagatose (lactose based) or fructo-oligosaccharides (sucrose based) have found applications in these products.

Specialized carbohydrates are used for the diabetic market to help control glycemic response. These can include carbohydrates that are somewhat resistant to enzymatic digestion, reducing the rate of monosaccharide delivery to the bloodstream, and carbohydrates that induce a large viscosity change when acidified (as in the stomach) and thus slow GI passage and digestion rates. Finally, some forms of enzyme inhibitors are at least under development; these would alter the body's ability to rapidly digest and absorb carbohydrates. This could be somewhat difficult to deliver from a practical point of view because there is a fairly wide variability in digestive enzyme levels, even in normal healthy adults, which can be exacerbated in the presence of certain disorders.

A wide variety of fat systems are used in these products. Usually they are derived from vegetable sources for the majority of the fat calories, and supplemented with sources of mono- and polyunsaturated fats (certain seed and marine oils). The goal is caloric enrichment without substantial cholesterol addition and avoidance of highly saturated fat systems. However, the products include reasonable levels of unsaturated fats with attention paid to appropriate levels of specific kinds of polyunsaturated fatty acids (PUFAs). Reasonable levels of omega-3 and omega-6 fatty acids and appropriate concentration

ratios are increasingly recognized as being important for optimal health.

Incorporation of acceptable levels of vitamins and minerals is also important, especially if the product is intended as a sole source of nutrition. A typical target is to include a daily supply of these key nutrients (depending on target country/regional regulations) with 1,000 to 1,500 calories of product energy.

Product Forms

As with infant formulas, product forms include liquids which are commercially sterilized via convection retorting or aseptic filling. Retort processing involves dispensing the products into hermetically sealed containers and then heat treating them, typically via water and steam combinations. Initially, metal cans were used almost exclusively for retort processed products. Now it is commonplace to find both glass and plastic containers, which are retort or thermally sterilized via ultrahigh-temperature-short-time (UHTST) and aseptically filled into sterile containers. Aseptic applications are attractive because they have a minimal effect on product color and nutrient thermal degradation.

Liquid products can be produced in ready-to-use or condensed forms, typically requiring a one-for-one dilution (though these are becoming rare, even in infant applications.) Condensed forms provide reduced storage and transport costs, both for the manufacturer and the end user, but they absolutely require distinct labeling to inform the caregiver that the product must be diluted prior to consumption. These concentrated forms also require a clean source of water for dilution purposes, as do the powder products. This is an important consideration for product use in a number of areas in the world today.

Products also are marketed in powder form, providing the most economical benefits to the consumer (both in cost per kcal and in storage and portability). Ease of reconstitution is important, which might dictate the types of proteins and carbohydrates used. Specialized final processing steps to aid in eventual dispersion of the product with water are also applied. Agglomeration is one such treatment; it produces a larger particle size, ultimately providing better initial wettability of the powder and aiding in the production of a smooth dispersion. Inclusion of polyunsaturated oils, while of significant potential physiological benefit, can limit flavor stability of powders after the sealed package is opened.

Satisfying the needs of the consumer requires attention not only to the nutritional content of the product, but also to its flavor and odor. Often there are advantages to delivering supplemental nutrition in a variety of forms (liquids, powders, bars, gels) to avoid flavor and consistency fatigue.

One other form of nutritional delivery is sometimes applied in both critical care and long-term care situations in which patients may have physical or mental barriers to normal oral food consumption. Food can be provided via a pump and a tube which allows delivery of liquid nutritional product directly to the stomach or even to the beginning of the small intestine (jejunum). This method reduces or completely eliminates the need for flavor and odor control of the product, though there might be some issues with flavor sensation from burp-up in conscious patients.

Typically, this type of product is specifically filled into ready-to-hang plastic containers ranging from 500 ml to 1 or 1.5 liters. The product is sterilized, either via retorting in these containers or ultra-heat treatment, prior to aseptic filling. Although products may demonstrate some phase separation during storage in these containers over their usual 12- to 15-month shelf lives, they become homogeneous suspensions after shaking and during tube feeding. Such feedings can last for up to 24 to 36 hours for each container, and thus the delivery package and/or system must maintain acceptable sterility during such extended delivery times.

The potential for product reflux back to the container is another packaging consideration. This can substantially contaminate the product with gut-associated microorganisms, ultimately reducing the product use (or hang) time; it may even potentially be dangerous to the patient. Products intended for tube feeding vary widely in caloric densities and nutrient distribution, depending on the needs of the patient. They must be stable in their original container and also in the container that is used during tube feeding (if that is different).

Some products are thin bodied and need enhanced viscosity to prevent phase separation. Viscosity can be increased by careful selection of the protein system, use of multivalent cation minerals, and/or addition of stabilizers. High concentrations of multivalent cations or stabilizers can cause gelation and unsightly phase separation. Appropriate product viscosity is not just an issue of mouth feel and pouring characteristics; it also can seriously affect swallowing performance (especially in patients with impairments such as dysphagia, in which case thick products are desirable) as well as tube feeding performance.

The necessity to deliver consistent nutrition through a small diameter tube also places restrictions on product viscosity, prevention of phase separation during feeding, and the absence of particulates (such as cream or sediment chunks) in the finished product. A number of actions can be taken to control viscosity in tube feeding formulations, which becomes more critical in high-caloric density (and especially in high-protein content) systems. Relatively low levels of hydrolysis of the protein system or use of insoluble mineral salts to deliver mineral requirements instead of soluble salts can reduce viscosity significantly. Although insoluble salts (particularly of multivalent cations) help to prevent increased viscosity, they also can form sediment sheets during product storage and may present difficulties during tube feeding. Insoluble fiber also tends to settle during tube feeding, so soluble fibers may be better suited to these applications. Ultimately, it is the careful blending and adjustment of all of these factors which results in an acceptable product.

Sports/Performance Nutrition

For serious athletes, a high-protein diet (preferably one that is also rich in branched-chain amino acids, particularly leucine) is considered very desirable. Leucine is known to be involved in priming skeletal muscle to receive meal-derived nutrients, particularly amino acids and sugars. A high-protein diet can supply appropriate building blocks for rapid muscular development and satisfy the elevated maintenance requirements in high-performance athletes.

In sports nutrition products, end user concerns about overall rapidity of delivery of nutrients, especially protein, and the muscle impact of specific amino acids (branched-chain amino acids) has led to a preference for whey protein. Whey protein is rapidly digested. It is highly soluble and does not form a curd in the stomach, which allows rapid stomach emptying. Furthermore, it is somewhat higher in overall branched-chain amino acid content than other typical nutritionally acceptable proteins such as casein and soy, though this content difference is not profound.

The physiological situation is more complex than is currently understood by the typical consumer. It is theoretically possible to overload the skeletal muscles' ability to absorb amino acids, which results in their use to produce energy or fat—both undesirable outcomes for dietary protein. Sports nutritional products also can be fortified with purified free amino acids to achieve a variety of end objectives, though this is somewhat limited by objectionable flavor and aroma characteristics, at least in some cases such as leucine and methionine. Based on digestion

characteristics, casein is considered a slow protein, whey is a fast protein, and soy is intermediate between these two. It is important, however, to note that relatively rigorous processing, as well as the presence of substantial fat and carbohydrates (which is very often the case in meal-type products), may alter digestion rates. Some also consider that the individual differences between these proteins are reduced or even eliminated by such processing and delivery. Further experimentation is needed to address these questions.

Alternate Forms of Nutritional Products: Bars and Gels

Broad use of semi-solid technologies has not occurred, although nutritional bars and puddings are currently widely popular, and protein-rich gels have started appearing in sports nutrition and "shooter" products, which deliver a relatively large dose of one or a few key ingredients in a very small volume of gel or liquid. Gels and puddings are typically delivered by violating one or more of the considerations described above for viscosity control, or by adding a gelling agent such as one of the pectins or a modified starch. Whey proteins in particular can be manufactured to produce gels. Defatted and undenatured whey protein isolates are used in high-acid drinks, creating a near clear solution. Whey protein hydrolysis also increases solubility.

High protein levels are obtained in nutritional bars via use of protein crisps, which do not create the shelf hardening issues observed with direct use of protein powders during product formulation. Crisps can contain up to 50% protein before hardness and graininess appear. In standard bars, protein can be delivered as part of a nougat or dissolved or suspended in a binder syrup. Hydrolyzed proteins or proprietary blends of intact and hydrolyzed proteins are used. Hydrolyzed proteins often have significant flavor issues, and masking the bitterness associated with their use is often very challenging; however, they do make a softer bar. The general difference in delivering protein in bars is that bars are usually discontinuous phase products, comprised of the filler, crisps, fruit or candied inserts, liquid fillers, and one or more types of coatings. There are commercial examples of inclusion of significant protein content in virtually all of these components.

Bar hardening associated with proteins is due to the terminal sulphur groups on the amino acid residues forming covalent bonds. In doing so, their net average molecular weight is raised, increasing their glass transition. Bar hardening may still occur with hydrolyzed proteins, but the smaller starting molecular weight causes it to take longer to reach the critical molecular weight that causes hardening. Judicial use of hydrolysates can result in an acceptable shelf life target. Hardening also can be minimized in non-crisp applications using glycerin and sorbitol, although sorbitol levels must be controlled due to its laxative effects. Glycerin has no laxative effects, but there are bitterness issues at certain product- and formula-dependent levels.

Technologies for Emerging Nutritional Needs

Physiological Functionality

Physiological functionality can be delivered by dairy-derived ingredients. One example is the use of lactoferrin to limit infection by limiting undesirable microbial growth via sequestering iron and by virtue of a substantial fragment, lactoferricin, which is a direct antibiotic.

Low but perhaps significant quantities of highly biologically active proteins are found in milk, including some very powerful growth factors such as insulin-like growth factor, transforming growth factor β (TGF-β), and immunoglobulins which function in nature to

passively immunize the young until they develop their own competent immune systems. Use of these types of ingredients is in the early stages. They currently are limited by their high cost and relative lack of resistance to heat treatments.

Colostrum, an early-lactation-stage material produced the first week or two of lactation after the calf is born, is another highly active milk-related ingredient. It is rich in immunoglobulins, growth factors, lactoferrin, and protein content, and very much lower in fat and carbohydrate content than mature milk. It is currently used as a specialty nutrition ingredient in health foods and sometimes in sports nutritional products. It is more expensive than mature milk due to its limited availability and seasonality in most places.

Some of the major proteins in cow's milk contain sequences of amino acids, which are at least theoretically released during the digestion process and confer their own biological activities. The presence of these sequences has been known for some time and they are appearing in products that are available as dietary supplements or even foods for special use. Chief among these is the use of protein hydrolysates rich in angiotensin converting enzyme (ACE) inhibitors. ACE controls one of the key mechanisms for regulating blood pressure. A number of commercial products available in the United States, parts of the European Union, Japan, Australia, and some other countries make blood pressure claims.

Because the structural requirements for ACE inhibitors are relatively small, it is not surprising that most proteins, if heavily hydrolyzed, have been found to be rather potent ACE inhibitors, at least *in vitro* and many *in vivo* (in both animal models and in humans), as well.

There are other sequences with demonstrated morphine agonist (casomorphins) or antagonist (casoxins) activity that may function in the control of gut peristalsis, though this is not clearly demonstrated. Such manipulation might be useful in maladies in which slowing the rate of food transiting the gut might be beneficial, such as short bowel syndrome. There is also at least one sequence with fairly well-demonstrated anxiety-reducing effects (α casozepine). A thorough review of the many potentially bioactive peptide sequences that are possibly available through the digestion of bovine milk proteins is beyond the scope of this chapter. Several good reviews have been published (see the references at the end of the chapter).

Overall, it is clear that there are many physiological impacts designed around the use of these constituents (either direct or through intensive digestion) that may be exploited, though only a few have been commercialized to date. This remains an area of potentially high commercial and health impact.

A variety of adult nutritional products enriched with specific amino acids, specialty oils, antioxidant and anti-inflammatory ingredients, high levels of specific vitamins, and so forth are available and widely used in specialty situations. Additional products range from those designed as general nutrition enhancements, to nutritional supplements for specific chronic disease situations, to the very specialized products designed for people with critical needs.

Table 20.5 gives some of these indications along with citations demonstrating clinical efficacy. The table is not intended to be complete in either identification of all of the many target populations addressed by such products, nor in the citations demonstrating efficacy. In most cases it cites a single example. The intention is to illustrate the breadth of applications currently available and suggest the huge potential for future improvements in coverage and the formulation to address specific patient group issues.

It is clear that there are a wide variety of reasonably accessible, sophisticated nutritional products designed for people with specialized needs. This product set is growing

Table 20.5. Medical nutritional product example categories.

Category	Typical nutrient content	Comments	Example clinical reference
General nutrition	Approximately 15%–30% protein, 20%–40% fat, and 30%–65% carbohydrates (by calories). Roughly 25% RDA of vitamins and minerals is typical target. Some antioxidant vitamins higher to enhance product stability	Products used as supplements to normal diet, as meal/snack replacements to support weight loss efforts, and as temporary sole nutrition sources	Products generally marketed on features, not generally studied clinically. Conforming to good nutritional guidelines and often containing some specialized ingredients to enhance immune status, protein nutritional status, etc.
Diabetes	Similar to general nutrition products, but characterized by higher-end fat content and/or specialized carbohydrate blends designed to extend duration and reduce magnitude of glycemic blood response	Products used to provide people with diabetes meal/snack alternatives with low glycemic response. Available in a number of different forms, including meal and snack bars, shakes, cereals, and puddings.	1. Broadhurst CL et al. (2006) *Diabetes Technology & Therapeutics* 8:677–687; 2. Yakamoto K et al. (2006) *Nutrition* 22:23–29; 3. LichtensteinAH et al. (2006) *Circulation* 114:82–96; 4. Katan MB et al. (2003) *Mayo Clin Proc* 78:965–978
Renal disease, pre-dialysis	Similar to general nutrition, but characterized by low protein content to reduce renal load	Products generally available as shakes or shake mixes	1. Zarazaga A et al (2001) Clinical*Nutrition* 20:291–299; 2. Burrowes JD, et al (2005) *J. Am. Diet. Assoc.* 105(4):563–572; 3. A Clinical Guide to Nutrition Care in Kidney Disease, Eds. Byham-Gray and Wiesen, from the Renal Dieticians Dietetic Practice Group of the American Association and the Council on Renal Nutrition 2004, Chapter 3
Renal disease, dialysis	Similar to general nutrition, but designed to replenish nutrients lost during dialysis, control blood glucose fluxuations, and generally provide higher protein content than the pre-dialysis products. Typically low in specific ions, especially phosphorous, potassium, calcium, and sodium.	Usually designed to control fluxuations in blood content and avoid undue kidney stress while replenishing nutrients lost to dialysis	1. Caglar K et al. (2002) *Kidney Int* 62(3):1054–1059; 2. Wolever T et al (2002) *Can J Diabetes* 26:356–362; 3. Wheeler ML, et al (1991) *Diabetes Care* 14:769–771; 4. Wolf BW et al *Nutr Res*2001;21:1099–1106; 5. Livesey G (2001) *Br J Nutr* 85:S7–S16
Long-term care	Usually general-nutrition-like products, which are used as oral meal supplements or as tube feeding products (with different packaging) to supply a nutritional gap up to sole source of nutrition	Generally the low-tech offering, characterized by nutritional adequacy in macronutrient and vitamin/mineral delivery in 1,000–1,500 kcal, usually offered in several ready-to-hang tube feeding options, mainly differing in planned volume of feeding and with limited oral sip feed packaging as well	These products are usually sold on features, again not generally studied clinically, but conforming with dietary standards for long-term sole source of nutrition. Typically contains 100% RDA of vitamins and minerals
Critical care	Wide variety of products available. Generally includes a base product (same or similar to long-term care offering) as well as a variety of specialty care products. Can include products supplemented with high levels of PUFAs, antioxidants, glutamine, arginine, etc. for the special impact these nutrients can provide	High-tech nutritional products, usually backed by moderate to extensive clinical testing and characterized by enrichment in highly bioactive component(s)	1. Singer P et al (2006) *Crit Care Med* 34(4):1033–1038; 2. Pontes-Arruda A et al (2006) *Crit Care Med* 34(9):2325–2333; 3. Cai B et al (2003) *Nutrition* 19:229–232; 4. AngelilloV et al (1985) *Ann. Intern. Med.* 103:883–885; 5. Brown RO et al (1994) *Pharmacotherapy* 14: 314–320; 6. Henningfield MF et al (1993) *FASEB J* 7(3):A377; 7

(continued)

Table 20.5. Medical nutritional product example categories. *(cont.)*

Category	Typical nutrient content	Comments	Example clinical reference
Cancer	Products aim to deliver enhanced protein content and calories to help in anorexia, and can include a variety of ingredients (e.g., enriched in polyunsaturated oils, high levels of branched-chain amino acids) aimed to blunt cachexia (unintended weight loss mainly from muscle driven by presence of tumors)	Products usually ready to use, mildly flavored, and relatively calorie dense. Usually designed to be delivered cold (which can help, indirectly, with treatment side effects such as mucositis)	1. Ryan AM et al (2009) *Ann. Surg.* 249:355–363; 2. Guarcello M et al (2006) *Nutr Ther & Metab.* 24:168–175; 3. Fearon K et al (2003) *Gut* 52:1479–1486; Barber M et al (1999) *Br J Cancer* 81:80–86
Performance Nutrition			
Protein shakes and powders	Major aim is delivering significant protein. Products range from simple flavored protein powders that consumers use to build customized shakes to relatively complete nutritional ready-to-use products, typically delivering high protein and low or no fat content	Achieving target protein intakes without exposure to higher than desirable saturated fat and cholesterol is difficult, and these products provide high supplemental protein to achieve high-performance sports goals	1. Wolf RR (2000) *Am. J. Clin. Nutr.* 72(2):551S–557S; 2. Lum C et al (2007) *Australasian J. Ageing* 26(4):168–172; 3. Burke D et al (2001) *Intl. J. Sport Nutr. Exer. Metab.* 11(3):349–364
Energy drinks	Sometimes built around hydration, providing carbohydrates for energy, and sometimes containing protein (most often whey)	More specialized offerings can include creatine (enhance energy storage), carnitine (enhance fat-burning capacity), taurine and specific amino acids, as well as many other ingredients	1. Welsh RS et al (2002) *Med. Sci. Sports Exer.* 34(4):723–731; 2. Ivy J et al (2002) *J. Appl. Physiol.* 93(4):1337–1344; 3. Campbell C et al (2008) *Int. J. Sport Nutr. Exerc. Metab.* 18(2):179–190; 4. Green AL et al (1996) *Am J Physiol* 271(5 pt 1):E821–E826
Diet support products	Typically modest calorie offerings, little or no fat, sometimes enhanced with satiety-enhancing ingredients (e.g., fiber, high protein, induced viscosity carbohydrate systems, etc.). Products are also designed around a particular diet program, such as high protein or a particular macronutrient balance	Product intent is to provide a healthy snack or "lite" meal, and newer entries add help with hunger signals to reduce difficulty with maintenance of hypocaloric diet	1. Tate DF, et al. (2006) *Arch Intern Med.* 166:1620–1625; 2. Apovian C et al (2009) *ICAN: Infant, Child & Adolescent Nutrition* 1:37–44; 3. Anderson JW et al (2004)*Advances in Therapy* 21(2):61–75

and can have a significant impact on the quality of life of people at risk for serious and limiting health issues. This category will continue to grow and become increasingly sophisticated as the population ages, our understanding is improved, and our interest in dealing with comorbidities increases. This is especially true in the areas of diabetes care, impact of obesity, and cardiovascular disease.

The Future

A wide variety of potentially industry-changing developments are being pursued at present, some of which we will mention.

Readers are encouraged to peruse their research libraries or the Internet to derive further information on these and the many other possibilities that exist both now and in our imaginations.

One example is designer whey products, with a range of flavored whey proteins and dairy calcium. These may provide a dietary protein boost and the benefits of dairy calcium, including both calcium homeostasis for bone and muscle health as well as the somewhat controversial impact of these minerals on fat metabolism.

Digestive problems are among the top four health concerns of consumers worldwide, according to the New Nutrition Business Report, 10 Key Trends in Food, Nutrition and Health (DII April 2009, page 30). A sense of a digestive "feel-good factor" is growing and will be reflected more often in product design, especially around protein sources.

Concern about overweight also ranks as one of the top four health concerns among consumers. A wide variety of efforts are aimed at this issue, including weight management through manipulation of satiety, use of active fiber, high protein and texture ingredients used in combination to slow digestion and movement through the gut, and use of specific inhibitors of digestive enzymes to reduce or even virtually eliminate digestion, especially of fats. Even more specialized ingredients can target weight loss to fat while sparing muscle and organ tissues. A substantial increase in the variety and technical sophistication of products aimed at this issue is expected. Many health care professionals view this as the top world health issue.

The use of protein nanoparticles (especially using denatured whey protein) to encapsulate and protect highly sensitive ingredients or bioactive compounds is an area of growing interest. Such encapsulated materials would be delivered as a result of digestion of the protein coating, which also would provide protein nutrition. These nanoparticles are mainly stabilized by disulphide bonds. This is analogous to the development of alternative packaging solutions (edible or highly biodegradable packaging).

The microencapsulation of probiotic organisms is a key area for technology development. Enhanced stability of the microorganisms, delivery of encapsulated probiotics in liquid ready-to-use product forms, and timed release are all current technology targets. Smaller capsule size results in greater palatability due to the reduced grainy mouth feel. Any technology must be food approved, which provides some limitations, but the potential benefits will continue to drive new development.

A product development goal is to learn more about the emulsion droplet interface. Particularly, controlling the amount of protein adsorbed at the droplet's surface (protein load) should modulate the properties of the emulsion. Control of product properties such as oxidative stability, kinetics of flavor release, or texture of rennet and acid gels are interesting targets for the immediate future.

Product Functionality

Physical functionality can be expressed within several contexts. Ease of ingredient manufacturing, ease of final product processing, and shelf life stability are three major areas in which dairy ingredients' functionality is important. The ingredient manufacturing aspects are covered in previous chapters. As the dairy proteins become more concentrated, the ability to disperse the proteins in the product process system can present challenges.

Manufacturing Considerations

A host of manufacturing questions encompasses a wide range of potential issues

common to all nutritional products that contain dairy proteins:

- How well do the ingredients mix together?
- Is there a unique sequence of mixing them that promotes better physical stability of the formulation?
- What are the ideal temperatures of mixing?
- What is the optimum pH or acceptable pH range?
- Do the proteins incorporate a lot of air during mixing, requiring deaeration prior to homogenization?
- What is the ideal homogenization pressure?
- Is additional heat treatment required, such as ultra-high-temperature-short-time?
- Does the protein(s) foul the equipment?

If the product is to be retorted, the mixing, pH, and processing requirements can have huge effects on the product's retort and shelf stability. One the other hand, if the product is to be spray dried, its concentration and the selection of carbohydrates during liquid processing can substantively influence the final powder quality.

Examples for Manufacturing Considerations

The following are some examples of the manufacturing questions, above.

- Typically, the proteins are blended together with the fat or oil, minerals, and some or all of the carbohydrates. Some proteins hydrate well in water, whereas others require high-shear mixing for total dispersion. With caseinates, especially calcium caseinate, prior dry blending with a carbohydrate helps disperse the caseinate particles and aid in their dissolution and hydration. In some cases, a protein-in-oil suspension is the preferred processing.
- As has been noted, native whey proteins are heat sensitive, while caseinates are not.
- There can be an optimum pH region for maximum heat stability with milk or products primarily comprised of milk. The ionic strength and concentration of cations (primarily divalent) can influence heat stability and the pH optimum region dramatically.
- For long-term shelf stability in liquid products, typically the higher the homogenization pressures, the better. The goal is to have the fat globules as small as possible to prevent the natural rise (creaming) in sterilized product over time. However, the protein must not only adequately cover the fat globules, but provide some charge to cause electrostatic repulsion of the other particles. Without adequate electrostatic repulsion, the protein-covered globules will adhere to each other, forming a distinct cream or sediment layer depending on the amount of protein covering the globules and their resulting buoyancy.
- Ultra-high-temperature-short-time (UHTST) treatments are an excellent way of reducing microbial counts, and can be instrumental in binding the whey protein to casein to afford the whey protein greater retort stability.
- Protein fouling on equipment can be a great nuisance and a significant cause of unacceptably short continuous process run times. Fouling is termed as either type A or type B. Type A is the building up of a proteinacous layer that restricts heat transfer and product flow. Type B is a mineral build-up that can be difficult to remove by CIP (cleaning in place) procedures, but otherwise does not affect processing. If fouling is problematic during process development, its resolution could involve prior preheating or forewarming the protein mixture, better balance of the minerals such as ratio of citrates and phosphates to soluble magnesium and calcium ions, and selection of the proper pH. It rapidly becomes clear that resolving processing

issues often involves alterations in either the product or the manner in which the product is put together.

- Products that are spray dried may create problems if the fat is not adequately homogenized; coating of the dryer or plugging of the cyclones may occur. Selection of emulsifiers can be critical as well. Because the product itself is not exposed to high temperatures, heat effects are usually minimal. Maltodextrins and milk proteins can interact, resulting in particles that are difficult to dissolve, which is a product acceptability issue.

Conclusion

This chapter is intended to serve as a brief overview of key aspects of this field of specialized nutrition—it is not a comprehensive treatment. While already large both in scope and in its impact on the human condition, the field of dairy ingredients in infant and adult nutrition is poised for a huge increase both in sophistication of the health impact and in further exploitation of the unique properties of milk. It appears that the ability to very specifically target key physiologic systems and enable targeted nutritional interventions that approach the realm of pharmacy is on our doorstep. The growing needs of public health in such areas as cardiovascular disease, obesity, and diabetes seem to perfectly fit the application of existing knowledge of preventative active nutrition, with a huge potential impact. It is truly a very interesting and rewarding time to be involved in this field, where a very old (but still not quite fully understood) source of early nutrition can be seen as a panacea of interesting and physiologically active food ingredients, just at a key time of need. While technological hurdles of supply, processing, and packaging may stand before some of these possibilities, it seems clear that we will derive ever growing benefits from milk.

Acknowledgement

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